# OXYGEN CONSUMPTION AND CHANGES OF COLOUR IN THE HUMAN EPIDERMIS: THEIR MEASUREMENT *IN VIVO* WITH THE CYCLOPS OXIMETER

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The cyclops oximeter was first developed by Brinkman and his colleagues to measure directly by reflection the level of oxyhaemoglobin in the skin capillaries. One needs no blood samples for this determination. Changes in haemoglobin saturation, not obvious to the naked eye, can be observed and recorded continuously by this method. Because the readings are commonly taken by fixing the photocells to the middle of the forehead, this type of electric eye was named the cyclops.

It works in the following way. The skin is kept flushed with arterial blood. Light reflection from sources in the skin other than oxyhaemoglobin are allowed for, and then the changes in skin reflection from a red light source are measured. As the blood is reduced, less red light is reflected. The photocell output is measured on a galvanometer, and logarithmic conversion gives the change in percentage oxygen saturation. An account of its development and construction is given by Zijlstra.[13]

While developing diverse techniques for measuring the reduction of haemoglobin *in vivo*, Brinkman observed and studied a peculiar phenomenon in the oxygen consumption of human skin. His earlier work on this subject, in which he used transmission oximetry, was more fully set out in 1942,[2] following an account submitted in 1939. It was later confirmed and extended by Lamberts,[11] who used the cyclops oximeter; and a short account afterwards appeared in English.[3]

Before this phenomenon is described, there are some features of skin respiration that must be mentioned. It is frequently anaerobic through pressure, it has no oxygen reserves, and it has a very low quotient of oxygen uptake. The circulation through the skin provides mainly for heat loss, and only a fraction of the blood that passes through it is needed for metabolic exchanges. It is therefore possible to observe changes in the rate of oxygen consumption in the skin from changes in tissue oxygen tension. In tissues that metabolize rapidly, the oxygen tension is kept so low that variations in the blood-oxygen level cannot be shown to alter it. In the fact that the skin differs from other tissues in these respects, we may find one possible reason for the phenomenon to be described.

Brinkman's phenomenon is this: When a human subject breathes pure oxygen for 10-20 minutes, cutaneous respiration is depressed. It is a progressive change and depends on the length of time that the pure oxygen is breathed. The phenomenon is seen *in vivo* whether the respiration is measured by transillumination or by reflection oximetry. The changes in respiration rate are readily measured in the skin of the finger, and are judged by the rate and extent of colour change in the blood after the circulation at the base of the finger has been arrested.

Colour changes other than those of haemoglobin can also be studied, and I have adapted the oximeter to one such investigation on melanin.

## INVESTIGATION

Prof. O. V. S. Kok, Department of Anaesthetics, University of Pretoria, kindly placed his cyclops oximeter,* at my disposal. Some potentialities and limitations of the instrument were investigated. Details of its normal operation are given by Zijlstra,[14] and are not repeated here.

### A. Studies on Direct Pigmentation (pigment darkening)

The cyclops oximeter is designed for use on white skin, and its application to negroid skin will obviously raise special problems. Nevertheless, we have found the oximeter useful in the Bantu for measuring differences in skin colour caused by changes in melanin pigment. Some of these I have reported.[7,8]

The following limitations were noted with the instrument.

*Made by P. J. Kipp & Sons, Delft, Holland.

A necessary step for reflection oximetry is a measurement of the reflection from bloodless skin. This is obtained by measuring the reflection through a plate of lucite, by means of which the skin is made bloodless through compression. On the Bantu skin it can readily be seen that compression of a degree past the point needed to make the skin bloodless produces a considerable difference in the reflection from the skin, doubtless because compression redistributes the skin components. As a result, it was found necessary when measuring direct pigmentation to use no compression whatever. This meant that a selective heat erythema in the skin has particularly to be avoided, since one must assume an equal circulation in the test and the control areas before the pigment change alone can be compared. In the aforementioned studies this limitation was not found to be serious, provided a large number of measurements were taken both in and around the skin site tested for its colour change.[7]

A more serious limitation, which is unrelated to the instrument itself, is the lack of what might, by analogy with haemoglobin, be called an oxygen-dissociation curve of melanin, as determined by reflection. From figures I obtained by the use of the Hardy reflectance spectrophotometer it is obvious that, when melanin is reduced with dithionite, readings in the 400–480 m$\mu$ range are useless for recording the colour change, 490–680 m$\mu$ reflection wavelengths are progressively more useful, and the changes between 680 and 750 m$\mu$ are parallel. These last showed a 15% increase in reflection from an excised specimen of Bantu skin that was fully reduced with dithionite.

Adapting these values to the Kipp oximeter and using a white tile for 100% reflection, I reported some values of full oxidation and full reduction of melanin.[8] With the Ilford filter No. 281, transmitting from 620 m$\mu$ upwards, I found on reduction a 12% increase in reflection.

I also reported a reflection drop of 20% on oxidation, but this was probably excessive for the following reasons. Chromium trioxide was used to effect oxidation of melanin previously reduced by dithionite; it was chosen because naked-eye comparison showed that it produced the darkest melanin. I now recognize that some of the dark colour may have been produced by chromic oxide in the tissue, which would make the reflection figure too low.

I have made some observations (unpublished) on the chemical oxidation of epidermal melanin in epidermal strips. On measuring the oxygen uptake of epidermis from air during chemical oxidation in the Warburg apparatus, it appears that from full reduction to full oxidation somewhat more oxygen is consumed per mg. dry weight of Bantu epidermis than is used for respiration when weak oxidants are used for the melanin.

The practical advantages of the Kipp oximeter in measuring direct pigmentation were used to compare changes in skin colour accurately on successive days, when only one variable was knowingly altered between one day and the next. In this way we were able to show that direct pigmentation could not be influenced in the Bantu by even the very highest blood levels of vitamin A.

### B. Electrical Reliability of the Cyclops Oximeter

In Zijlstra's most recent book on oximetry[14] he makes no mention of the properties of the photocells as a source of experimental error, possibly because this touches the suppliers rather than the experimenter. The following investigations led me to question the suitability of the photocell type used in the construction of the apparatus. In these studies, I was privileged to have the theoretical and practical guidance of Mr. J. de Jager, electrical engineer, during January and February 1963:

*1. Analysis of the Multiplication Factor*

The cyclops photocell is of the selenium blocking-layer type, and when the external resistance placed across it is changed the galvanometer is supposed to give a ten-times higher reading. These two choices of resistance setting are called 0·1 × sensitivity (Ro·1) and full sensitivity (Rc) in the instrument as sold. All the resistances in the switchbox of the instrument we used were of values that produced the expected change, and the galvanometer deflection produced by a constant current across the photocell input was increased virtually ten times when the smaller resistance was switched into the circuit. There was clearly no defect in our switchbox.

*2. Measurement of the Multiplication Factor with the Photocell in the Circuit*

Having already had reason to doubt the constancy of the multiplication factor with the photocell in circuit, I raised the question in July 1961 with Professor Zijlstra. He replied that it is also not possible simply to determine the sensitivity ratio between Rc and Ro·1—the factor always equals 10, which is the responsibility of the factory.

The following simple tests led me to conclude that the sensitivity ratio was not only easy to determine with the photocell in the circuit, but that the factor was sometimes a long way off what it was supposed to be.

With new photocells installed by the factory in 1961 and stored correctly, the cyclops was placed on a series of 9 variously coloured surfaces (red to brown) and the red reflection measured on each, both at one-tenth and full sensitivity. All variable circuit rheostats were kept at maximum resistance throughout (extreme left). At full sensitivity care was taken to test practically two full galvanometer scale lengths (400 mm.), which cover the range of colour change between fully oxygenated and fully reduced blood in the skin. The protocol most favourable to the instrument was as follows:

| *Colour* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* |
|---|---|---|---|---|---|---|---|---|---|
| Rc | 197 | 149 | 97 | 48 | —1 | —50 | —100 | —149 | —197 |
| Ro·1 | 121 | 116 | 110 | 103 | 96 | 92 | 87·5 | 84 | 78 |

By taking these 9 pairs of values 2 at a time, the multiplication factor between the lower and higher pairs can be calculated. The results were tabulated accordingly as follows:

| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
|---|---|---|---|---|---|---|---|---|
| *9* | 9·2 | 9·1 | 9·1 | 9·8 | 10·9 | 10·5 | 10·2 | 8·0 |
| *8* | 9·3 | 9·3 | 9·1 | 10·4 | 12·5 | 12·3 | 14·0 | |
| *7* | 8·9 | 8·7 | 8·8 | 9·0 | 10·7 | 11·1 | | |
| *6* | 8·3 | 8·3 | 8·2 | 8·9 | 12·7 | | | |
| *5* | 13·2 | 7·5 | 7·0 | 7·0 | | | | |
| *4* | 8·3 | 7·8 | 7·0 | | | | | |
| *3* | 9·1 | 8·7 | | | | | | |
| *2* | 9·6 | | | | | | | |

It can be seen that the multiplication factor varied from 7 to 13, and generally lay somewhat below 10. Disconnecting the posterior photocell confirmed the impression that the

multiplication factor was consistently somewhat below 10. These findings were laid before the manufacturers, who replied: 'With regard to the variations found by you it can be assumed that new photocells will not give improvement in this matter and we keep with you to the opinion that the cells in question still are in good working order'.

*3. Other Sources of Variable Readings*

Random short-term variations of up to 15 mm. at full sensitivity on the galvanometer were also met with. The makers' comment on this was: 'Random short-term variations can always be expected with selenium cells. When these cells are in constant use these variations usually decrease gradually'.

Before suspecting the photocells we had felt obliged to exclude every other easily testable source of error in our instrumental technique. Dry test surfaces were used and at constant temperature; stray light was excluded, and the built-in stabilizer was found to correct completely for random mains fluctuation of the grossest kind.

With photocells over 2 years old, the multiplication factor was greatly influenced by the rheostat regulating the compensating current, when the instrument was running at full sensitivity. The multiplication factor was found to vary by anything from 1 to 6 times, according to the strength of the compensating current. Most probably the main fault here was a deterioration in the front photocell. I tried to correct this by filtering the light stimulus to the compensating cell, but this merely moved the galvanometer spot to the right without correcting the lowered multiplication factor. Even if the front photocell were in good order, the manufacturers have suggested a circuit modification of the posterior compensating photocell to minimize the faults revealed by my tests. So far we have not put their advice into practice.

*4. Galvanometer Adjustment*

When consecutive readings are being taken over more than one galvanometer scale range, I mentioned to Professor Zijlstra the possibility of reversing the terminals on the galvanometer to make available a second scale. He replied that a fuller scale was secured in his laboratory by rapidly transposing the zero to the right during the experiment (the values are always falling anyhow). Having inserted a simple household tumbler switch in the leads to the galvanometer, I find this a better method of range extension with the existing apparatus.

*5. Theoretical Considerations*

An explanation for these vicissitudes in photocell behaviour can possibly be found in the variable activity of barrier-layer photocells under high external resistance such as is required by the circuits of the cyclops oximeter. According to Van Geel,[12] selenium cells give a linear output independent of temperature with white light at about 10 ohms external resistance, but this changes to a non-linear temperature-dependent curve with very little current production as the external resistance rises to 1,000 ohms and upwards. At a resistance of 4,000 ohms (approximately that of the cyclops instrument) the voltage changes measured will not differ much with small changes of total resistance provided the illumination is low and covers a small range of the curve so as to be virtually linear. Any alteration beyond this narrow range will destroy linearity. When asked if another type of photocell could be used, the firm replied: 'In view of the circuit as used in the cyclops it can hardly be realized to apply for example a Phillips vacuum phototube instead of the selenium cell. We are sorry that we cannot give you advice in this sense.'

**C. Oxygen Consumption rate of Skin** *in vivo*

*1. Effects of Croton Oil*

It has been shown that croton oil inhibits aerobic cell respiration, possibly by blocking the respiratory chain somewhere between cytochrome b and cytochrome c. This action may have something to do with its action as a co-carcinogen in experimental animals.[1]

For our experiments croton oil BPC, was used, as supplied in South Africa in Lennon's series of Dutch medicines. Ultraviolet spectrophotometry of a 3% solution in Merck's hexane showed that it had a marked absorption in the ultraviolet, increasing greatly from the visible down to the sunburn range. Its slight effect on an oxidative system of tyrosine and tyrosinase I have reported earlier.[9]

Using auxiliary apparatus built by the technical department at our University, almost exactly like that of Lamberts,[11] in June 1961 Dr. Geoffrey Falkson and I tested the effect of croton-oil painting on the oxygen consumption of the skin. After preliminary flushing induced by histamine iontophoresis, normal desaturation graphs were made, followed by painting of pure croton oil to the dorsum of the finger being tested; the excess was lightly dabbed off. In my own case as test-subject, a single normal control curve was made at zero time followed by croton-oil painting at 10 minutes and control curves at 30 and 50 minutes. No obvious differences were seen between them. In Dr. Falkson's case, 6 consecutive desaturation curves were plotted over a period of 90 minutes, pure croton oil having been applied directly after the first test. Again there was little difference between the graphs. A curve 19 hours after croton-oil painting again gave the same result.

These entirely negative results may depend on a number of technical differences in method. Thus, it is conceivable that dilute croton oil in hexane would penetrate better than pure croton oil. This is hard to accept if hexane evaporates quickly, as indeed it would do on the skin. It is also conceivable that our samples of croton oil were lacking in co-carcinogenic effect. As long as such a co-carcinogenetic property eludes isolation, the investigator of its action is usually expected to join the losing side if he publishes negative results. Another variable is the time lapse between the application of croton oil and the measurement of respiratory inhibition. Allison and Lightbown[1] waited 4 hours when using mice, but then also added croton oil to the mince of the painted mouse ears in the Warburg apparatus. In this way the previous painting of one ear each of the mice used loses its significance as a procedure and makes the time interval of no account. In our study we could not have waited much longer than $1\frac{1}{2}$ hours, in which time the results were negative, because the histamine flush would by then have subsided. One last possibility is that mouse and human epidermis react differently to croton oil as a co-carcinogen and respiratory inhibitor (see below).

*2. Effects of Pure Oxygen Inhalation*

With a photocell that did not give full multiplication, curves of colour change in the finger were recorded which fell within a 50–60 mm. range. On several trials for the effects of breathing pure oxygen for 4–15 minutes on desaturation, identical curves were obtained before and after. In two trials the finger was kept flushed by histamine, and in the remainder

flushing was accomplished by reactive hyperaemia. Again no differences were noted in the curves.

## DISCUSSION

There are several problems that hinder one's acceptance of the Dutch work on this subject. Some of these are now presented.

When the decline in oxyhaemoglobin is measured by reflection, it is noteworthy that the curves as published by Brinkman and Lamberts are hyperbolic, and not sigmoid or in any other way similar to the oxygen dissociation curves of haemoglobin. This might perhaps be due to the influence of superimposed diffusion factors between the capillaries and epidermis, but it remains unexplained.

Oxygen uptake in the epidermis has been shown in several species to increase with moderate elevations of the oxygen tension,[4] and this is the reverse of one's expectations, were Brinkman and Lamberts' findings correct. There seem to be three possible choices open. First, the human epidermis may act differently to raised oxygen tension from that of animals. Second, raised $0_2$ tension may alter diffusion in the dermis without changing cell respiration. Third, the Brinkman effect may be a technical artifact, on the side either of those who hold that it exists or those who deny it.

Next, the inhibition of skin respiration by ultraviolet light as described by Lamberts is open to question. He used an energy of $3{\cdot}46 \times 10^3$ erg/sec.cm.$^2$ at 360 m$\mu$. This is a remarkably low dose. Dr. I. A. Magnus, of the Institute of Dermatology, London, tells me that the most severely light-sensitive patient he has tested so far gave no skin reactions (minimal erythema, etc.) to monochromatic light at 360 m$\mu$ with energies less than $10^7$ erg/sec.cm.$^2$ This apparent discrepancy has still to be clarified.

Despite these considerations which tend to throw doubt on the findings, a number of topics would repay investigation if the difficulties could be overcome. Some of these follow:

The Brinkman effect would help greatly in explaining the mechanism of direct pigmentation. I have proposed a hypothesis[10] that direct pigmentation is brought about by the oxygen made available through suppression of cell respiration from long-wave ultraviolet light.

The Brinkman effect from pure oxygen can also be simulated by traces of ozone in the atmosphere.[3] Underground miners with many years of service under conditions of artificial ventilation may possibly be exposed to such a hazard. In South Africa it has been shown by Erasmus[5, 6] that they develop a severe type of scleroderma. In this disorder a respiratory poisoning by ozone may contribute, though silica dust probably has an obscure part to play as well. I raised this question with Professor Brinkman, but apparently the mines in Northern Europe have not presented him with such problems.

Our failure to reproduce the Brinkman effect may be the result of the preceding anaemic occlusion of the skin circulation. Perhaps breathing pure oxygen has some threshold effect on the tissues before the phenomenon of depressed respiration occurs. We also feel that unedited curves over a small range are more likely to mean something than recalculated values that trespass into the range of greater instrumental variations. Possibly too the subjects were breathing traces of ozone unwittingly during the control period from the water-pump motor.

## SUMMARY

The cyclops oximeter is a potentially excellent tool for analysing certain dermatological data such as intermittent day-to-day colour changes as well as continuous changes over short periods. Sundry problems of a technical and interpretative kind are bound to arise when the machine is used. An account is given of its application to the measurement and interpretation of direct pigmentation, the measurement of ultraviolet damage to skin respiration, the action of co-carcinogens, and the pathogenesis of scleroderma in miners.

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