A method for the measurement of transmitted and reflected light from the same biological sample

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Summary. An apparatus is described for the measurement of transmission and reflection of visible light through biological materials. The accuracy of the apparatus is independent of any fluctuations of intensity in the light source.

Recently, there has been an ever-increasing interest in light and its effect on living matter. One could hardly conceive of a form of life that is not light dependent. Light can be beneficial or deleterious in its effect on living tissue. The protective effect of melanogenesis as well as the harmful cancerogenous effect in skin due to solar radiation is well known. The ability of light to penetrate many tissues has been reported in the literature[1-4]. Lately, light has been used to treat newborn babies who are afflicted with neonatal jaundice. Light which penetrates the skin destroys the bilirubin circulating in the capillaries of the skin, thereby avoiding the necessity of resorting to the more complicated procedure of exchange blood transfusion[5].

Skin color is influenced by solar radiation. The darkening of the skin due to increased production of melanin, following exposure to sunlight, is an every-day occurrence. The color of the skin influences the absorption of solar radiation and this, in turn, at least in some animals, has been shown to affect the energy economy of the entire body. Birds painted black and exposed to artificial sunlight used an average of 22.9% less energy[6].

The melanocyte-stimulating hormones (MSH) produced by the pituitary gland and the melatonin produced by the pineal gland have a definite darkening and lightening effect, respectively, on the skin of some species. Isolated pieces of frog skin have been used for the quantitative assessment of these darkening and lightening agents. Measurements of transmitted or reflected light are indicative of the shielding power of the tissue to visible light and the ability of some agents (MSH, melatonin, caffeine, Acth, etc) to change the skin[7-9]. Light striking the skin may be reflected, scattered, transmitted, or absorbed. Usually, different apparatus are used to make transmission and reflection measurements and quite often small variations in the voltage of the power supplied to the light source adversely affect the precision of the measurements.

It is the purpose of this paper to describe a new apparatus which is capable of measuring light both transmitted and reflected by biological tissues. A photometer is also described which uses a reference photocell which avoids the necessity of precisely controlling the source of illumination.

Photometer circuit. A problem commonly encountered in making transmission and reflectance measurements is the instability of the light source. Slight variations in the voltage across the lamp have a pronounced effect upon the intensity of the source, making it absolutely necessary that the voltage be carefully regulated. The modified Wheatstone Bridge circuit shown in figure 1 was used to obviate this difficulty. A reference photocell and a measuring cell (Clairex CL 905 HL photocells were used for each) have been placed in the circuit. Since the 2 photocells always receive light from the same source at the same intensity, an ordinary light bulb and an inexpensive voltage source may be used.

Transmission measurements. To make transmission measurements, the light source (figure 2) is placed at the center of an optical bench. The reference and measuring photocells are placed on either side of the lamp housing so that each photocell is 200 cm from the center of the source. To balance the photocells, the relative values R_A and R_B (figure 1) are adjusted to provide a null reading on the galvanometer. With a sample in front of the measuring cell, its photocell carrier must be moved closer to the source to receive the same intensity of light as the reference photocell which is maintained at 200 cm. Since the lamp may be assumed to be a point source, the inverse square law can be used to calculate the percent transmission. The apparatus was calibrated by using a series of Kodak Wratten No. 96, neutral density filters, of known transmission (±5% of the nominal value) which were placed in the specimen holder. The data presented in table 1 were obtained.

The application of the inverse square law. To balance the apparatus, the measuring photocell carrier is moved closer to the light source until the intensity of the light transmitted through the specimen (I_{200}) is equal to the intensity of the light reaching the reference cell. When a balance is obtained, the measuring cell is at a distance, d, from the light source while the reference cell is maintained at 200 cm. The percentage of light transmitted by the specimen is then given by:

$$\% \text{ Trans} = \frac{I_{200}}{I_d} \times 100$$

but

$$I \alpha \frac{1}{d^2}$$

Therefore,

$$d = 200.0 \text{ cm} \sqrt{\frac{\% \text{ Trans}}{100}}$$

Reflection measurements. To make reflection measurements, the measuring cell holder is mounted on the base of figure 2 by passing the adjusting post of the housing through the slotted hole in the measuring cell holder. (It should be noted that 3 translational and 3 rotational adjustments have been provided to permit the precise positioning of the sample.)

Table 1. Calibration of the photometer measuring transmitted light

Neutral density filter (nominal % Trans)	Theoretical distance of measuring cell from source (cm)*	Actual distance of measuring cell from source (cm)**
100 (No filter)	200.0	200.0
80	178.9	176.6
63	158.8	157.3
50	141.5	141.5
40	126.5	126.4
32	113.1	111.5
25	100.0	99.5
20	89.5	91.1
16	80.0	77.7
13	72.1	71.6
10	63.2	63.1
1	20.0	20.1

*$d = 200.0 \text{ cm} \sqrt{\frac{\% \text{ Trans}}{100}}$.

**Reference cell maintained at 200 cm from the source.

A front surface mirror (virtually 100% reflection) is inserted into the slotted specimen frame holder, and the cell carrier is carefully adjusted to reflect the light in such a way that a circle of light is centered on the photocell opening. Since the total distance from the lamp to the mirror, and then back to the measuring photocell is made exactly 20 cm, the moving reference cell is placed 20 cm on the opposite side of the lamp, and the bridge is balanced. (A null reading is

Table 2. Calibration of the photometer measuring reflected light

Neutral density filter (nominal % reflect)	Theoretical distance of reference cell from source (cm)*	Actual distance of reference cell from source (cm)**
100 (No filter)	20.0	20.0
80	22.3	22.6
63	25.2	25.7
50	28.2	28.5
40	31.6	32.2
32	35.3	36.7
25	40.0	41.5
16	50.0	52.6
13	55.5	57.2
10	63.3	65.1
1	200.0	230.0

$*d = \frac{20.0\text{ cm}}{\sqrt{\frac{\%\text{ Reflect}}{100}}}$. **Measuring cell maintained at 20.0 from the source.

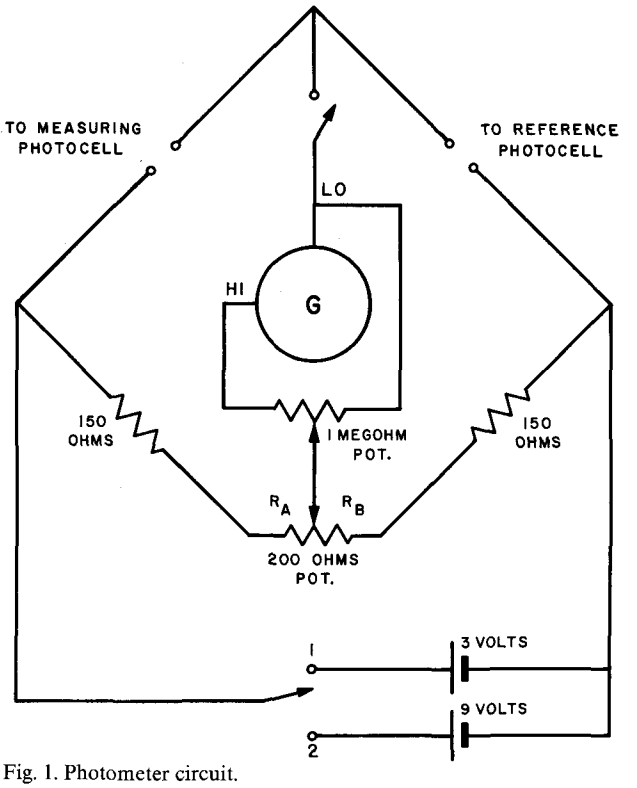

Fig. 1. Photometer circuit.

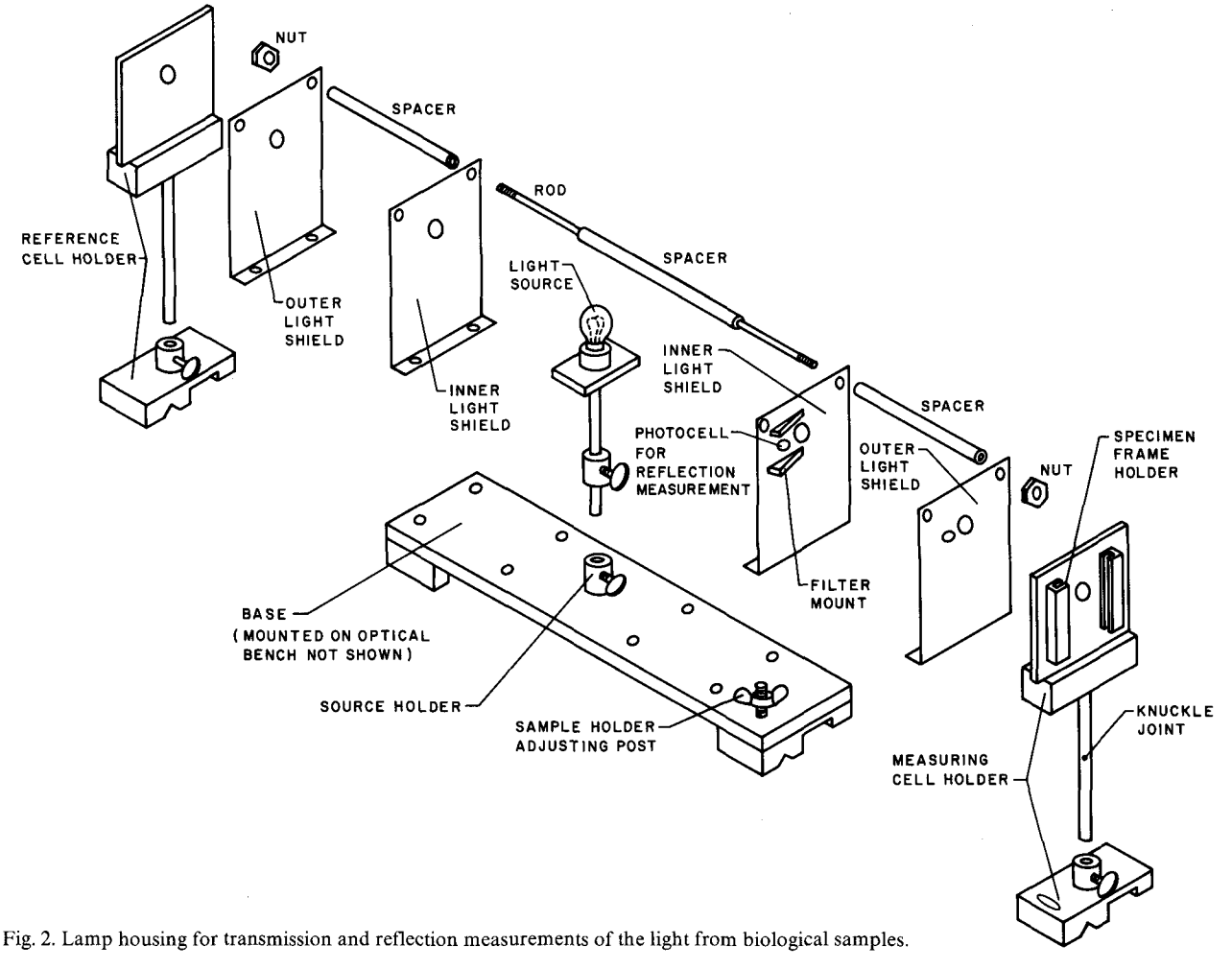

Fig. 2. Lamp housing for transmission and reflection measurements of the light from biological samples.

obtained by adjusting the ratio R_A/R_B.) The mirror is removed and the specimen frame containing a sample of the skin being tested is inserted into the cell carrier. Since the intensity of the light reflected (the angle of incidence of the light to the sample is approximately 10° from the normal) from the specimen is much less than that reflected from the mirror, the moving reference cell must be moved away from its original position to receive light of the same intensity as the measuring cell. By measuring this distance and assuming that the lamp is a point source, the percentage reflection may be calculated.

To calibrate the apparatus, the mirror was reinserted into the cell holder and a neutral density filter of known transmission was inserted into the filter mount in front of the mirror. The data presented in table 2 were obtained. Reflection values below 1% were obtained by inserting a 25% neutral density filter in front of the moving reference cell. To calibrate the apparatus, other neutral density filters were then placed in front of the mirror and the data presented in table 3 were obtained.

Specimen frame. Specially built frames were used to hold the samples. They were made of aluminum and were blackened. The frame for the skin consisted of 2 plates with the overall measurements of 5 × 5 cm. The first plate had a ridged hole in its center (1 cm inner diameter) and 2 guide pins for positioning: one in the upper left corner, and the other in the lower right corner of the plate. The top plate had a large hole in its center (1 cm inner diameter) and 2 smaller holes to match the pins of the bottom plate. Once the skin was spread over the ridged hole of the bottom plate, and gently stretched, the top plate was pressed firmly in place and held by 2 spring clips. The specimen frames were made to fit snugly in the slotted frame holder so that the sample was in line with the light source and the photocell. Furthermore, positioning was such that the beam of light always impinged upon the same spot of the sample in all subsequent measurements.

Conclusion. The apparatus described in this paper has been used to investigate the variation of light transmitted through the skull and scalp of the rat, following drying[10]. Small variations in transmissions were measured over a wide range of values. Precision is limited, mainly, by the care exercised in aligning the apparatus. Transmission measurements may be made from 1% to 100% while reflection values ranging from approximately 0.1% to 100% are easily obtained.

Table 3. Calibration of the photometer measuring light less than 1% reflected

Neutral density filter in front of measuring cell (nominal % Reflect)	Theoretical distance of reference cell from source (cm)*	Actual distance of reference cell from source (cm)**
25	20.0	20.0
16	25.0	26.3
13	27.7	28.7
10	31.6	32.4
1	100.0	112.0
0.1	316.0	325.0

$$*d = \frac{20.0}{\sqrt{\frac{\%\ \text{Reflect}}{25}}}.$$

**Measuring cell maintained at 20.0 cm from the light source and 25% filter in front of the moving reference cell.

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On a rabbit hyperlipemia induced by a fungic galactomannane peptide

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Summary. I.v. injection into rabbits of a fungic galactomannane peptide isolated from the culture medium of *Aspergillus oryzae* induced the apparition, 20 h later, of an hypertriglyceridemia, with a concomittant decrease of about 70% of the post-heparin lipoprotein lipase activity. The same effect had been obtained earlier with a carbohydrate-rich fraction purified from a crude papain preparation. The 2 fractions are compared.

It was shown in previous experiments that the i.v. injection into rabbits of some glycoproteins[1-3], glycopeptides[4] or pituitary extracts of peptide nature[5-7] was followed by the apparition of hyperlipemia. In this study, similar results have been obtained by the injection of a fungic galactomannane peptide (FGMP) isolated from the culture medium of *Aspergillus oryzae* (IP 410). We have compared this new result with the hyperlipemia, induced in rabbits, by the injection of a glycopeptide A1 extracted from a crude papain preparation[4].

Materials and methods. The fungic galactomannane peptide (FGMP) was extracted from a culture medium of *Aspergillus oryzae*[8] (strain IP 410, type UF 3981) provided by the Rapidase Society, Seclin, France). The dialyzed culture medium was centrifugated at 6000 rpm for 20 min, and the supernatant filtered through a column of hydroxyapatite according to the method of Benardi and Kawasaki[9]. The FGMP fraction was eluted with a 0.005 M pH 6.8 sodium phosphate buffer[10]. 3 mg of this fraction were injected into the marginal vein of rabbit's ears (Fauves de Bourgogne).

The sugar determination of the FGMP and A1 fractions, the measurement and the ultracentrifugation of the serum lipoproteins, and the estimation of the post-heparin lipoprotein lipase activity (PHLA) of the rabbit post-heparin