BRIEF REPORT

Quantitative Studies on the Flicker Phenomenon in the Erythrocytes

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THE FLICKER PHENOMENON in the erythrocytes is difficult to describe or to define. It is well known that the normal erythrocyte has the shape of a biconcave disk with a pale center and, when living, exhibits two different activities: first, vibratory variations of density inside the cytoplasm, best visible at the junction of the outer dark and the pale central regions; second, slow undulating movements of the membrane resulting in the formation of irregular bulges which appear and disappear by intervals.

The flicker phenomenon was first described in 1890 by Browicz,[1] using ordinary light microscopy. In 1901, Cabot[2] confirmed this observation. In 1949 Pulvertaft[3] described the flicker as seen with phase-contrast microscopy which makes it very prominent. According to this author, the phenomenon is present in mammalian, avian and amphibian erythrocytes; it is also present in reticulocytes, abnormally shaped anemic erythrocytes, in erythroblasts, but not in proerythroblasts, nor in "ghost" cells. Pulvertaft mentions that fluorides inhibit the flicker in normal cells and suggests that this phenomenon may be related to glycolysis. In 1950, Blowers et al.[5] made an extensive study of this problem; they found that the flicker is inhibited in the presence of fluorides and mono-iodo acetate, but persists in the presence of carbon monoxide, cyanide and azide. They concluded that it is related to glycolysis but not to respiration. Parpart and Hoffman[5] in 1956 contradicted these conclusions entirely. According to these authors, the flicker phenomenon is simply the result of Brownian motion. In order to clarify these discrepancies, it seemed important—as an initial study—to obtain quantitative records of the phenomenon permitting measurement of its frequency.

According to the literature, only few attempts have been made in the past to quantitate the flicker. Technics which have been used include cinematographic recording,[6] stroboscopy[3] and television microscopy,[5] but none of these experiments have been successful.

We assumed that the best way of recording and eventually quantitating

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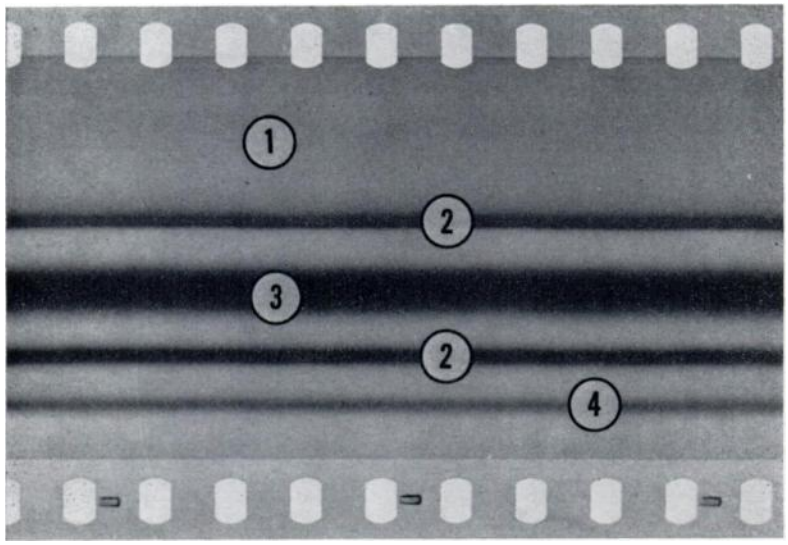


Fig. 1.—Sample strip of film showing the appearance of continuous records. 1.—background (intercellular plasma); 2.—margins (plus phase halo) of the erythrocyte; 3.—center of the erythrocyte; 4.—margin of another erythrocyte not completely visible in this field.

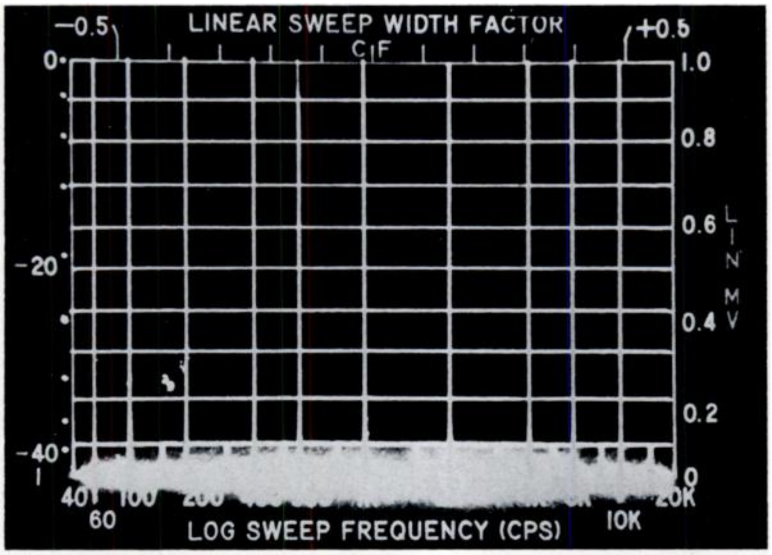
the flicker was to use a continuous recording technic, e.g., magnetic tape recording, or recording on continuously transported motion picture film. This can be achieved by technics which are very similar to sound recording. Playing back such records in conjunction with a suitable electronic analysis equipment should permit quantitation.

Materials and Methods

In the experiments which shall be described here, all preparations of fresh blood were made in a very simple way. A small drop of normal blood, taken by puncture of a fingertip, was placed on a microscope slide and simply covered with a coverslip. All observations were made in phase-contrast microscopy at 37 C.

Continuous Motion Picture Film Recordings

Various commercially available 35 mm. "oscilloscope" cameras were used, but all gave unsatisfactory results because of nonlinear film transport. As a consequence, a specially designed camera was built, which shall be described elsewhere in detail.[7] This camera is similar to an optical sound recorder. It uses 16 mm. film and has proven very satisfactory in terms of speed stability and linearity of film movement. With this machine, records were made in the following way. Using only the microscope optics, the image of an erythrocyte to be recorded is projected onto the film plane. The film moves at a constant speed of 20 perforations per second behind a transverse slit. This slit, 1 mm. in width, is positioned in such a manner as to record the central portion of a single erythrocyte. After processing, the film shows traces (Fig. 1) which correspond to (1) background, (2) erythrocyte membrane and (3) central region of the erythrocyte. The illumination of the microscope was provided by incandescent lamps (Osram 12 V. 100 W. or CDF 100 V. 500 W.) fed with A.C. or D.C. In the case of A.C., as can be expected, a strong component of 120 C.P.S. was introduced in the record but did not interfere with analysis since it was easily recognizable. The film records were played back by



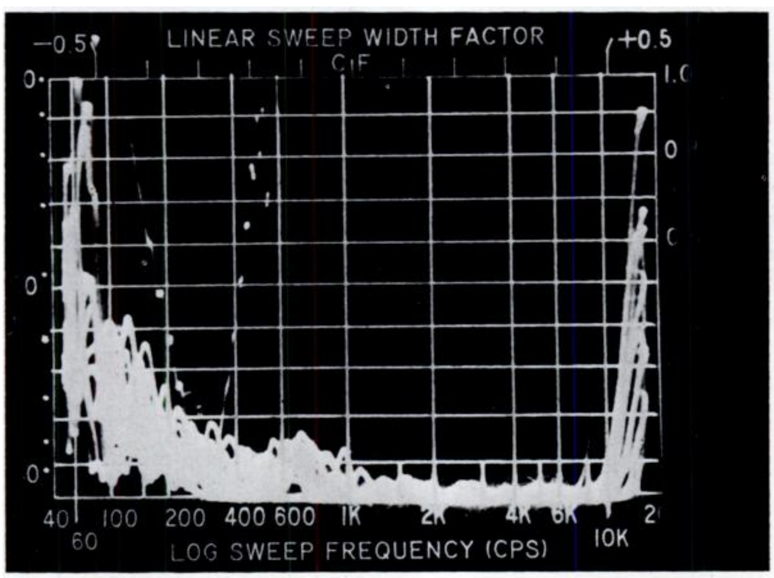


Fig. 2 (above) and 3 (below).—Photographs of the screen of the spectrum analyzer during play back of records. In Figure 2, the photofet was located in area (1) of Figure 1. In Figure 3, it was located on the edge of the central region of the erythrocyte. The "peak" which is visible at 600 C.P.S. is not significant: it corresponds to 120 cycles originated by the illumination system fed on A.C.

projecting them continuously with the same machine which was used for recording (provision is made in the machine to use it as a short distance projector) at the same speed of 20 perforations per second. The image was projected onto a small photo-sensitive field effect transistor (Crystalonics "Fotofet" FF400) about 5 mm. in diameter which could be accurately positioned in the areas (1), (2) or (3) shown in Fig. 1. Signals originating from this device were studied by means of a spectrum analyzer (Panoramic Sonic Analyzer LP-la). This instrument does not respond at frequencies below 20 C.P.S., and therefore the playback of the film records was speeded up. This was achieved by transferring them on magnetic tape (using a Honeywell 1/2 inch tape recorder) prior to feeding them to the spectrograph. In this way, it was possible to play the tapes at speeds which were known multiples (from 2 to 5 times) of the recording speed. A level where the spectrograph had a satisfactory response was reached and the original frequencies were calculated, after calibration of the equipment, by a simple division.

Magnetic Records

Attempts were made to obtain records by projecting the microscope image directly onto a photocell connected, through amplifiers, to the magnetic tape recorder. However, due to the relatively low energy of light coming out of the phase-contrast microscope, a high degree of electronic amplification was required (the tape recorder needs about 1 V. at the input) and this introduced a large amount of background electronic noise which made such records extremely difficult to interpret.

Results

About two dozen records were made and analyzed as previously described. They exhibited very constant results, two of them being reproduced in Figures 2 and 3. It can be seen that practically no "noise" appears in the background. The flicker, in the human erythrocyte, under "normal" conditions does not display any significant isolated frequency, but rather is made of a mixture of a large number of low frequencies varying from about 40 C.P.S. to 0.

Summary

A technic has been described which allows one to analyze the vibratory variation of density which is visible inside the cytoplasm of the erythrocytes and has been termed "flicker." Further investigations will be performed, dealing with variations of physical conditions and with the action of various chemicals in order to determine whether or not this phenomenon is related to a metabolic activity.

SUMMARIO IN INTERLINGUA

Es describite un technica que permitte le analyse del variationes vibratori de densitate que es visibile intra le cytoplasma de erythrocytos. Le termino "flicker" (scintillation) es proponite pro le phenomeno. Investigationes additional va esser effectuate pro clarificar le variationes occurrente in le "flicker" con variationes del conditiones physic e del effecto de varie compositos chimic, con le objectivo de determinar si o non iste phenomeno es relationate con un activitate metabolic.

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