

Rate of Escape of Haemoglobin from the Erythrocyte.

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The technical problem of finding the rate at which pigment leaves the mammalian red cell was proposed to me by Dr. Hugo Fricke, with the idea that if the rate were known it might be possible to calculate whether the pigment leaves the cell through one or two holes in the membrane, or by diffusing across the membrane generally.

The method which I have used consists in taking motion pictures of the haemolysing cells.* The best cell to work with is the red cell of man, which is relatively large. A suspension is made by suspending the thrice-washed cells from 4 cc. of blood in 20 cc. of 1% NaCl, for a suspension of this strength, when diluted with equal volumes of a lysin solution, shows from 5 to 15 cells per frame of the film used (35 mm.). Saponin made up in 1.0% NaCl in such a dilution as produces complete lysis in about 3 min. is a convenient lysin. The optical system used should be one of the highest resolution practicable, *e. g.*, an aplanatic condenser of N.A. 1.4 working at $\frac{2}{3}$ cone and critically focussed, a 1/10 inch oil-immersion objective of N.A. 1.37, and a 10x eyepiece. If the illuminant is an arc used without color filters, the exposure is about 1/30 second. A film speed of 16 frames per sec. is quite sufficient.

To 0.5 cc. of the suspension is added 0.5 cc. of the dilution of lysin sufficient to give complete lysis in about 3 min. A drop of the mixture is rapidly placed on a slide and covered with a coverglass smeared with vaseline along the edges. The vaseline seal prevents the cells from drifting. The preparation is placed on the stage of the microscope, and the cells focussed through a side telescope which enables them to be seen while the film is in a position ready for exposure. It takes about 30 sec. to make the preparation and focus the cells. The film is then exposed until most of the cells in the field, which can be seen and kept in focus all the time through the telescope, have haemolysed.

There are two methods of dealing with the finished film, which must be developed under strictly standard conditions. The first is to look at it in a machine which drives it at such a rate as to give

* I have to thank Dr. Robert Chambers for letting me use his motion picture camera, and I have been able to avail myself of the expert technical assistance of Mr. C. Grand.

the sensation of continuity, to fix one's attention of a single cell, and to take the time between the moment when it first begins to fade and the time when it appears a mere shadow with a pigment concentration about 10% of the initial. Since the footage which passes through the camera as the film is being made and also the footage which passes through the machine as the film is being observed are known, one can arrive at a very good idea of the time which the cell takes to haemolyse, and the fact that the machine can be reversed so as to give the appearance of the shadows regaining haemoglobin makes the observations all the more accurate.

Observations made in this way show that the loss of pigment is quite a slow process, occupying from 2 to 6 sec. This is very much slower than would correspond to the haemoglobin diffusing generally across a completely permeable membrane (*i. e.*, through water). There is no visual evidence that it leaves through a rent or hole, and, although the slow rate is consistent with this mode of escape, it is equally consistent with the pigment's leaving by diffusion across an injured membrane which permits less free diffusion than does water.

The second method of dealing with the film is to carry out densitometry on the film-images in every fourth frame, so as to determine the amount of pigment in the cells at intervals of 0.25 sec. This is a highly technical procedure. It is sufficient to say that the rate of loss of pigment from the individual cell is such as might be expected if the loss were roughly represented by an exponential function. The results, which are of little interest apart from their mathematical treatment, and also results (very similar in kind) obtained when other lysins and types of cell are used, will be reported elsewhere.

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Quantitative Nature of the Red Cell Response to a Single Bleeding.

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In the course of experiments designed to test for the presence of an erythrocytogenic hormone in the serum of rabbits subjected to bleedings,¹ a close correspondence between the number of red cells and reticulocytes of several control animals during the recovery

¹ Gordon, A. S., and Dubin, M., *Am. J. Physiol.*, in press.