A Photoelectric Method for Estimating the Pupillary Flow of Aqueous Humour

In a recent paper[1] a new method for measuring the pupillary flow of aqueous humour has been described. If the content of the anterior chamber is stained with fluorescein, introduced via the cornea (preferably by means of iontophoresis) and the dye is well mixed in the aqueous (by movements of the eye or by increasing the convection), the newly formed aqueous is seen as an uncoloured 'vesicle' emerging from the pupil. By a photogrammetric procedure, the size of the vesicle can be measured and an estimate of the pupillary flow can be obtained.

In order to eliminate the photographic processes involved in this method, it has been modified in the following way. If the eye is observed in a slit lamp microscope, where the slit is widened so far that its image covers the pupil, a 'cuvette', enclosing the pupil, can be formed by masking the image plane of the microscope objective lens in a suitable way (Figure 1). A photomultiplier cell, adapted in one of the binocular microscope tubes behind the masking, measures the amount of light emitted from the fluorescein in the 'cuvette', the other tube being used for searching purposes.

When the fluorescein concentration is relatively low, as in the present case, there is a linear relation between concentration and light emission. The photocurrent – which is continuously recorded as an ink-written curve – can thus be considered proportional to the amount of fluorescein in the 'cuvette' (apart from a constant amount of background light).

Starting from a well-mixed state, the fluorescein is seen homogeneously dispersed in the aqueous, but very soon clear aqueous appears at the pupillary border. A growing 'vesicle' is seen which after some time reaches the boundaries of the 'cuvette'. During this phase there must be a loss of fluorescent aqueous over the borderlines of the 'cuvette', exactly corresponding in volume to the gain of clear aqueous flowing from the pupil. Consequently, the amount of light emitted from the cuvette should decrease linearly, if we presume a constant flow. For the relative light intensity recorded at time zero, we then have:

$$I(0) = V c , \tag{1}$$

and t sec later

$$I(t) = V c - f t c , \tag{2}$$

where V is the cuvette volume, c the concentration of fluorescein, and f the flow (mm³/min).

When the newly formed aqueous more or less passes the boundaries of the cuvette, the fall in I is no longer linear, but becomes slower, and the slope (dI/dt) approaches the horizontal.

A calibration is introduced by shielding off a fixed small area of the aperture in front of the photomultiplier tube (Figure 1). Since the width of the slit beam, the angle between the slit projector axis and the axis of the microscope and the corneal curvature are known, the calibration means the exclusion of the light from a known volume, say V_{cal}. On the assumption that the calibration is performed in the initial well-mixed state we have

$$V_{cal}\, c = I_{cal} . \tag{3}$$

Eliminating V and c from the equations (1, 2, 3) we get

$$f = \frac{V_{cal}}{t} \frac{I(0) - I(t)}{I_{cal}} .$$

Since all magnitudes to the right are known or determined from the recording, the flow is easily calculated. In the determination of flow in absolute units, a minor correction should be introduced to make allowance for the effect of the corneal curvature.

Figure 2 shows a model experiment made in a glass cell containing a fluorescein solution and a source of constantly running clear water. The linear phase and the calibration effect are shown. The value calculated from the curve agrees with the known flow value.

The difficulties encountered in measurements of the living eye are caused by the need to fix the eye and avoid blinking during the short period needed for a measurement (15–20 sec). However, by mixing the content of the anterior chamber by moving the eye, a new starting position is obtained. Several measurements can be made in a few minutes. Figure 3 shows a curve recorded from a human eye. The flow value calculated falls inside the normal range[2].

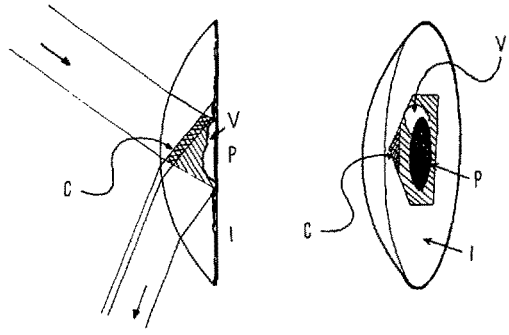

Fig. 1. Anterior chamber seen from above (left) and from the microscope (right). The measuring 'cuvette', limited by the light beam from the slit lamp and the masking in front of the multiplier tube, is striped. P, pupil; I, iris; C, calibration volume; V, clear aqueous.

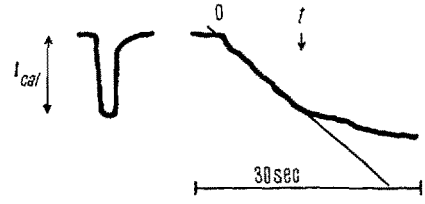

Fig. 2. Model experiment with glass cell containing a fluorescein solution and a source of clear water; showing a calibration and the decline of light intensity – in the beginning linear – with time.

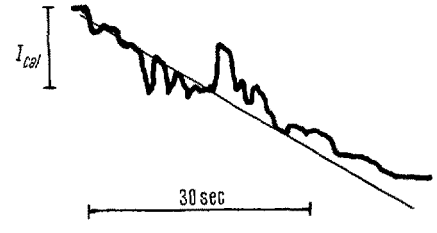

Fig. 3. Recording from a human eye. Though the course is disturbed by small eye movements, the declining trend is obvious.

[1] O. HOLM and C. E. T. KRAKAU, Experientia *22*, 773 (1966).

[2] This work has been supported by grants from the Swedish Medical Research Council.

Zusammenfassung. Im transkorneal mit Fluorescin gefärbten Kammerwasser verteilt sich der Farbstoff unter anderem durch Augenbewegungen gleichmässig. Aus der Pupille herausfliessendes, neugebildetes Kammerwasser ist ungefärbt. Photoelektrische Messung der Fluoreszenz einer kleinen, die Pupille einschliessenden Region ergibt Absinken der Fluoreszenz durch neugebildetes Kammerwasser. Mittels Kalibrierung lässt sich die Geschwindigkeit der Fluoreszenzminderung zur Berechnung der Minutenvolumina auswerten.

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Blood-Brain Barrier Damage and Prolonged Cerebral Hyperemia Following Changes in Cerebral Perfusion Pressure; an Experimental EEG Study

An adequate reduction of the cerebral perfusion pressure is usually followed by prolonged cerebral hyperemia[1]. The factors eliciting the hyperemia are still unknown. The duration of the hyperemia, however, far exceeds the calculated flow debt, indicating a damage to the autoregulatory mechanisms.

The aim of the present study was to evaluate a possible relationship between the reactive hyperemia and changes in the EEG and/or the blood-brain barrier function.

Methods. The experiments were performed on adult mongrel dogs under light nembutal anaesthesia. Cerebral blood flow was measured according to Lassen et al.[2]. Cerebral perfusion pressure was varied by infusion of a mock cerebrospinal fluid into the cisterna magna through a two-channel needle, allowing continuous registration of the induced CSF pressure. The animals were mechanically ventilated and had normal values of PCO_2 and O_2 saturation. In some animals the systemic arterial pressure and, accordingly, the cerebral perfusion pressure was raised by the i.v. injection of epinephrine and metaradrine (aramine). EEG was registered by means of 6 bipolar or monopolar leads via gold-plated stainless steel electrodes screwed into the calvarium. The EEG registration was carried out with an eight-channel Kaiser EEG apparatus. The blood-brain barrier function (BBB) was tested by i.v. injection of benzyl penicilline and of vital dyes (Evan's blue, fluorescine)[3,4].

Results. Figure 1 presents a typical experiment from a series performed on 6 dogs. (A) shows the resting EEG at normal systemic arterial blood pressure and cerebrospinal fluid pressure. 1,000,000 IU of penicilline/kg body-weight was injected i.v. with no obvious effect on the EEG during an observation period of 30 min (B). The cerebrospinal fluid pressure was then increased to 100 mm Hg, giving a marked reduction of the cerebral perfusion pressure, resulting in an isoelectric EEG during 2 min (C). After normalization of the cerebrospinal fluid pressure and thus also of the cerebral perfusion pressure in the EEG activity, when reappearing, was of a paroxysmal type (D), which continued for a considerable time. In non-immobilized animals symptoms of generalized seizures could be observed.

Another series of experiments, comprising 8 dogs, is represented by Figure 2. After i.v. injection of penicilline no effect was observed on the EEG during 30 min (B). Norepinephrine was then injected i.v., causing a rapid increase of the systemic arterial pressure. When the blood pressure reached a level of 240 mm Hg, paroxysmal activity appeared in the EEG (C), accompanied by generalized convulsions. The same effect was obtained after elevation of the systemic arterial pressure with aramine. Raising of the systemic arterial pressure without previous injection of penicilline did not cause convulsions or changes in the EEG.

Normally penicilline does not pass through an intact BBB[3-5]. The present findings suggest that an impairment of BBB function was the result of the reduction of the perfusion pressure. A further support for this suggestion was obtained by a diffuse staining of the cerebral parenchyma after intravital injection of Evan's blue and fluorescine.

It seems that a gross reduction of cerebral perfusion pressure as well as an adequate increase of the systemic blood pressure are of importance for producing lesions of the BBB in dogs and thus, in the presence of an epileptogenic factor like penicilline, for producing epileptic mani-

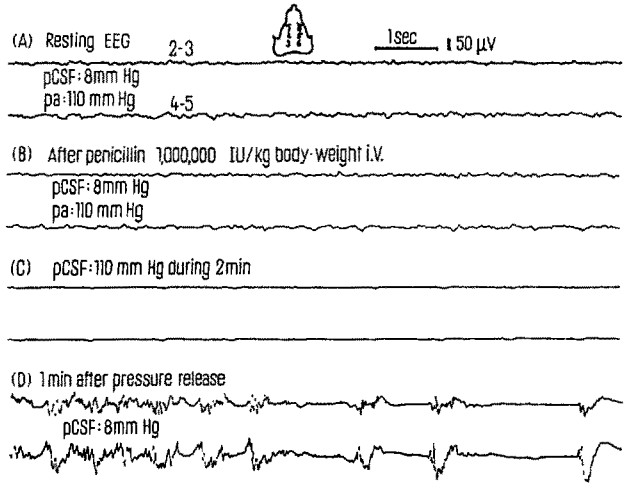

Fig. 1

[1] E. Häggendal, J. Löfgren, N. J. Nilsson and N. Zwetnow, Verh. Int. Neurochirurgen-Kongress, Bad Dürkheim (1966).
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[5] R. Gonsette, Acta neurol. psychiat. belg. *5*, 313 (1956).