Automatic recording of corneal thickness in vitro. STEPHEN D. KLYCE AND DAVID M. MAURICE.

An addition to the specular microscope is described which allows it to record the thickness of the excised cornea automatically as a function of time. The focus of the instrument is scanned mechanically through the tissue, and the position of the reflecting surfaces is detected by a photometric system and marked on a chart recorder. The system is able to follow thickness changes over periods of many hours and with an accuracy greater than obtainable by manual operation. This system has been helpful in the evaluation of a new medium which considerably extends the useful lifetime of the corneal endothelial fluid pump.

The specular microscope has been used extensively to measure the thickness of the excised and perfused cornea. Generally, readings are taken at intervals by a trained observer, which is an acceptable procedure when the preparation survives for only about 7 hours, as is commonly the case. However, several workers are attempting to find which are the missing factors which would extend its lifetime, and any degree of success results in inconveniently long working periods. It was evident that an automated system was called for, and this report describes an attachment to the specular microscope that has been developed for the continuous measurement of corneal thickness.

Materials and methods. Under the specular microscope, readings of corneal thickness are obtained by difference on manually focusing either up or down through the cornea and recording the calibrations on the fine-focus dial when a slit of light projected through the objective is focused on its surfaces. A reflecting surface forms an image of the slit at the eyepiece, and this image shifts across the field when the microscope is moved through focus. The position of the slit on an eyepiece scale proved to be a more reproducible indicator of the position of the surface than a subjective estimate of sharpness of its image.

Description of apparatus. In the automated version (Fig. 1) the focus of the microscope is scanned mechanically through the cornea while the positions of the surface reflections are sensed and recorded electronically. The rotary motion of the fine-focus dial is sensed by a ten-turn potentiometer coupled to the dial to provide a 10 mV. increment in signal for each 3.60° (equivalent to 1 μm) of rotation. This signal is fed through a sample-and-hold module to drive the Y axis of a 10" chart recorder (Model SR-255B, Heath Co.) while the chart is driven at slow speed (0.01" per minute). Motorized vertical scan (100 μm per minute) is accomplished by driving the fine-focus dial with a reversible 4 r.p.m. synchronous motor. The motor alternately raises and lowers the microscope body through an adjustable scan range of 50 to 950 μm. As the microscope is scanned through the cornea, the focused images are transmitted by fiber optics to a photometric system whose output triggers the chart recorder to make a dot (by lowering the pen) corresponding to the position of the fine-focus dial each time a peak of relatively intense reflection is sensed.

As mechanical hysteresis is inherent in the microscope, a double dotted line is traced for each reflecting surface when both directions of scan are recorded. To check for accuracy and stability, the thickness of a coverglass slip was...
Fig. 1. Schematic of the automatic specular microscope. The image of the reflected light slit is focused by the microscope objective (O) on the end of a fiber optics device (F.O.). The receiving end of the fiber optics is a 0.5 by 5 mm. rectangular aperture, which roughly corresponds to the dimensions of the focused slit. The scan motor is reversed with a precision level detector (RCA CA3098). Light intensity is sensed with a photomultiplier (RCA 931A) whose amplified and filtered output current can be displayed directly on a chart recorder (as in Fig. 2) or used to initiate the recording of the position of a reflecting surface. A discriminator which undergoes a transition only when peaks of adequate intensity are sensed is composed of a peak detector, a level comparator, and an AND gate. This transition activates two timers, one which commands the sample and hold module to store the current value of microscope position and a second which commands the recorder to lower the pen.

monitored continuously for 20 hours with less than 1 per cent variation in the range of recorded thickness (128 to 129 μm). The recorded thickness was within 1.5 per cent of that obtained by conventional (manual) operation.

Tissue preparation. Corneas from New Zealand White rabbits killed by overdose of sodium pentobarbital were mounted in the specular microscope as previously described. When both outer and inner surfaces of the cornea were perfused, a modified chamber was used.

The perfusion solution consisted of 50 per cent balanced salt solution with the addition of adenosine and reduced glutathione and 50 per cent of a complete culture medium plus gentamicin (25 μg per milliliter) as the sole antibiotic. This combination had been found to give longer survival than any other medium tried hitherto. Earlier lack of success with standard culture media can be attributed to the use of penicillin and streptomycin to ensure stability.

Results. Photometric scans through the isolated rabbit cornea are shown in Fig. 2. In the intact cornea with both surfaces perfused with medium, it is possible with a narrow illumination slit and high photomultiplier gain to detect reflections from the following interfaces: tear-epithelium (TE), epithelium-stroma (ES), stroma-Descemet's membrane (SD), and endothelium-aqueous humor (EA). This allows the thicknesses of the epithelium, the stroma, and the combined Descemet's membrane and endothelium to be determined simultaneously. With the epithelium scraped away and the outer stromal surface blocked with silicone oil (presently the standard procedure to assess activity of the endothelial fluid pump), distinct reflections are detected at the oil-stroma (TS) and at the endothelium-aqueous interfaces (EA). Irregular light scatter, generally of less intensity than the interface reflections, arises from stromal elements.

A sample experiment to test the adequacy of the perfusion medium in the extension of endothelial fluid pump lifetime is illustrated in Fig. 3. In this experiment the epithelium was scraped away and the stroma was swelled from that surface with perfusion medium. Subsequently, the outer surface was covered with silicone oil and the automatic measurements begun and carried on without interruption. The endothelium thinned the corneal stroma at a maximum rate of 55 μm per hour from an initial thickness of 515 μm to a minimum thickness of 382 μm, which was close to the in vivo level. Stromal thickness was maintained within 8 per cent of the minimum value for over 30 hours. A gradual swelling oc-
curred during the third and fourth days of incubation, with a maximum swelling rate of 15 μm per hour after 80 hours. During the latter part of the experiment, stromal light scattering increased in intensity, which obscured the focused image of the endothelium and reduced the reliability of measurements from that surface. At 81 hours a double reflection was recorded from the outer corneal surface which, on direct observation, proved to be the formation of a fluid layer between the oil and the stroma. At this time, stromal thickness was nearly twice normal, and consequently the swelling pressure would be about 10 mm Hg, which approximates the pressure maintained on the aqueous humor side. The fluid layer formed presumably because the stromal fluid pressure rose to a positive value.

In six additional experiments with the perfusion medium noted above, the endothelial fluid pump was found to preserve stromal thickness within 10 per cent of the initial value for over 30 hours.

Discussion. This is the first report of a device capable of automatic corneal thickness measurement. Automatic recordings of the transmission of light through the cornea have been accomplished previously, but light transmission is not a linear function of stromal hydration and is a relatively insensitive measure of thickness until this has increased some 70 per cent. In addition, total light transmission is not a function of stromal hydration alone, for epithelial edema can also diminish corneal light transmission. For these reasons, corneal thickness is best measured by direct means.

Automation of the specular microscope provides a method for accurately recording corneal thickness in vitro for long periods of time. Operator reading errors are eliminated, and therefore the accuracy of the determinations is generally improved. Furthermore, it is possible to separately measure the thicknesses of the epithelium and Descemet’s membrane–endothelium accurately, which has proved difficult with manual operation. The long-term stability of the device has been of assistance in determining the adequacy of a new culture medium to considerably increase the useful lifetime of the isolated cornea.

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REFERENCES

Fig. 3. Temperature reversal and subsequent swelling of an isolated rabbit cornea. In the upper panel are shown segments of the chart record produced by the automatic specular microscope. The upper double line of dots corresponds to the position of the stroma-oil interface; the lower series of parallel dots corresponds to the endothelial position. The difference between corresponding dots is plotted in a running time average in the lower panel.

Toward the latter part of the experiment the discriminator sensitivity was increased to detect the endothelium as overlying swollen stroma scatters more light, obscuring the endothelial reflection. Note the appearance of a second series of dots at the epithelial surface at 81 hours. This corresponds to the appearance of a fluid layer between the stroma and the oil.


Vertical striae in the posterior cornea were produced experimentally in ten human subjects by depriving the anterior corneal surface of its normal oxygen supply and inducing corneal edema. These striae were similar in appearance and time of occurrence to those observed in gel lens wearers. Three subjects also wore gel lenses and de-