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The Mechanism of Corneal Accommodation in Chicks

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Corneal accommodation can account for up to 9 D of accommodation in a freely behaving chick. We have explored the possibility that changes in corneal curvature are due to changes in intraocular pressure (IOP) during accommodation. In an *in vitro* preparation we demonstrate that increasing the pressure will tend to flatten the cornea. We have used electrical stimulation of the Edinger–Westphal (EW) nucleus to further test the pressure hypothesis *in vivo* by recording IOP changes in the eye during EW-stimulated accommodation and by artificially modulating the IOP to assess the effects on corneal curvature. During EW stimulation there is an increase in IOP on the order of 1–3 mmHg which tends to flatten the curvature of the cornea, thus eliminating changes in IOP as a possible mechanism of corneal accommodation. Slit-lamp observations of accommodative changes at the corneo–scleral margin and electrical stimulation of dissected eyes *in vitro* indicate that corneal accommodation is mediated by a contraction of the ciliary muscles, which exerts a pull on the inner lamella of the cornea, flattening the peripheral cornea and increasing the curvature of the central cornea. Histological examination of the ciliary region of the eye confirms the appropriate positioning of the ciliary muscles. We conclude that corneal accommodation in the chick eye is accomplished by a ciliary muscle-mediated mechanism.

Chick Intraocular pressure Ciliary muscle Edinger–Westphal nucleus Slit-lamp

INTRODUCTION

The cornea is the primary refracting surface of the eye in terrestrial animals, and in humans it can account for as much as 75% of the refractive power of the eye (Helmholtz, 1909). An ability to increase the curvature of the cornea affords some birds with an accommodative advantage beyond that of other terrestrial animals. Although Young (1801) demonstrated that accommodation in humans does not include a corneal component, in the literature concerning birds there exist both experimental evidence for corneal accommodation (Beer, 1893; Gundlach, Chard & Skahen, 1945; Schaeffel & Howland, 1987; Troilo & Wallman, 1987) and suggestions of its existence (Slonaker, 1918; Walls, 1967; Duke-Elder, 1958; Pumphery, 1961; Meyer, 1977; Martin, 1985).

Corneal accommodation has not been consistently found in all bird species and its very existence has been questioned. Cramer (1853) found no changes in corneal curvature when accommodation was electrically stimulated in excised pigeon eyes. Although Beer (1893) demonstrated changes in corneal curvature in a variety of bird species, he was unable to induce corneal accommodation in the domestic chick. Gundlach *et al.* (1945) measured changes in corneal curvature of up to 17 D in pigeon eyes following application of nicotine and curare. More recently, however, corneal accommodation was not observed in pigeons and mallard ducks when accommodation was induced with nicotine sulfate (Levy & Sivak, 1980) nor during electrical stimulation of chick eyes (Sivak, Hildebrand, Lebert, Myshak & Ryall, 1986). Behavioral studies on owls indicated the absence of corneal accommodation (Steinbach & Money, 1973). Schaeffel and Howland (1987) demonstrated that in freely accommodating chicks and pigeons, up to half of the full range of accommodation (15 D) can be accounted for by changes in corneal curvature and that in pigeons almost all of the accommodative range (9 D) could be accounted for by changes in corneal curvature. Troilo and Wallman (1987) showed that about 40% of the full range of Edinger–Westphal (EW) or nicotine sulfate stimulated accommodation could be accounted for by changes in corneal curvature.

Descriptions of the precise mechanism for avian corneal accommodation have been many and varied. The first experimental evidence for changes in corneal curvature during accommodation comes from Crampton (1813), who believed that a contraction of the anterior ciliary muscle would flatten the cornea as an accommodative mechanism for far vision. Brücke (1846) disputed this hypothesis in favor of the opposite view that central corneal curvature would be increased by a

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contraction of Crampton's muscle. Beer (1893) described the ciliary muscle as exerting its pull on the inner lamella of the cornea to flatten the peripheral cornea while increasing the curvature of the central cornea. Slonaker's (1918) morphological examinations of the eye of the English sparrow also led him to conclude that corneal accommodation is accomplished by a contraction of the ciliary muscle. In contrast to Beer, Slonaker suggested that a contraction of the ciliary muscle would decrease the circumferential diameter of the eye at the corneo scleral margin, increase the intraocular pressure (IOP) and thereby increase corneal curvature.

It has been suggested that accommodation in birds is mediated by changes in IOP (Hess, 1909; Slonaker, 1918). From morphological studies, it has been suggested that the anterior division of the ciliary muscle of the avian eye is particularly well adapted to alter the diameter of the corneo–scleral venus plexus (Suburo & Marcentoni, 1983; Tripathy & Tripathy, 1973) and change fluid pressures in the anterior chamber of the eye. Such a mechanism has also been postulated for the ciliary muscles of the eyes of diving mammals to compensate for environmental pressure changes during dives (Murphy, Bellhorn, Williams, Burns, Schaeffel & Howland, 1990). It might, therefore, seem possible that corneal accommodation in chick eyes could be mediated by changes in hydrostatic pressure behind the cornea.

A direct pressure-mediated mechanism of corneal accommodation has previously been proposed whereby a decrease in pressure during accommodation would cause the corneal radius to be reduced and thereby increase the refracting power of the eye (Romano, Schaeffel & Howland, 1989). Such a mechanism necessarily relies on sufficient elasticity of the cornea and a restrictive force applied at the base of the cornea. The scleral ossicles, a ring of bones embedded in the scleral matrix of the eye at the corneo-scleral region, could provide support at the base of the cornea, but it remains questionable that the cornea is sufficiently elastic to alter its radius of curvature under the normal range of physiological pressures. While stress–strain relationships of the chick cornea show it to have a lower modulus of elasticity than that of mammalian eyes (Romano *et al.*, 1989), which do not exhibit corneal accommodation, this fact alone is insufficient to show that the elasticity is adequate to account for corneal accommodation.

In vitro pressure changes in intact human and rabbit eyes have previously been used to study the elastic properties of the cornea (Jue & Maurice, 1986). To test if changes in corneal curvature could be accomplished by hydrostatic pressure changes in bird eyes, we have applied increasing hydrostatic pressures behind excised chick corneas while measuring the curvature of the corneas. We have also investigated the change in IOP in intact eyes *in vivo* during EW-stimulated accommodation and the effect of changes in IOP on corneal shape. Our *in vitro* and *in vivo* experiments show that the pressure changes normally observed during accommodation are insufficient in magnitude and are in the wrong direction to account for corneal accommodation, thus refuting an exclusively pressure-mediated mechanism. We provide evidence for a direct ciliary muscle-mediated mechanism of corneal accommodation from slit-lamp observations of EW-stimulated accommodation, electrical stimulation of enucleated eyes and histology. Preliminary reports of these findings have appeared elsewhere (Glasser & Howland, 1990; Glasser, Troilo & Howland, 1992; Glasser, Troilo & Howland, 1993).

METHODS

Subjects

Cornell K-strain White Leghorn chicks were obtained at hatching and maintained under a 12/12 hr light/dark cycle with *ad libitum* feed and water. In all the EW stimulation experiments described below, chicks were maintained under 40% urethane anesthesia (i.p., 0.7 ml/100 g). General anesthesia was supplemented with local subcutaneous injections of 2% lidocaine hydrochloride (Butler, Columbus, Ohio) 2–3 min prior to cuticular incisions or eye cannulations. Chicks were euthanized with an additional 0.7 ml/100 g injection of urethane or by using ether or CO_2.

Hydrostatic pressures applied behind excised corneas

Corneal curvatures were measured in three 10-week old chicks using infrared video-keratometry as described below. Keratometry was first done on the living birds under dim illumination (accommodation relaxed) just prior to euthanasia. To verify behavioral measurements of the range of corneal accommodation of the chick eye (Schaeffel & Howland, 1987), corneal curvature was measured 63 times in one chick accommodating to targets held at varying distances from the animal's eye. A least value for the possible range of corneal accommodation is given by the difference between the largest and the smallest of measures of corneal curvature.

Chicks were euthanized and the eyes enucleated and placed in physiological saline. The corneas, together with 5 mm of the surrounding scleral margin (containing the scleral ossicles), were dissected free and maintained in fresh saline until used. The tissues were clamped between two plexiglas plates, a base plate and a face plate, each with a 10–15 mm dia hole drilled through the center of the plate [Fig. 1(a)]. The hole in the face plate was bevelled on the inner surface to allow the scleral margin to rest in the bevel while the cornea protruded through the front of the plate. Several face plates with differing hole diameters and bevel angles were available to achieve the best fit of the scleral margin of the eye into the hole. A rubber washer was positioned on the posterior surface of the sclera between the tissue and the base plate and the two plates were clamped together with four set screws to create a water tight seal around the scleral margin. A hydrostatic column connected through a pressure transducer (Grass Instruments) was applied through the hole in the center of the base plate. By adjusting the height of the water column, a known hydrostatic pressure could be applied to the posterior surface of the cornea. The hydrostatic column was filled

with physiological saline and the anterior surface of the cornea was kept moist by the occasional application of a drop of saline.

The pressure transducer was connected to an A/D board of a PC via an oscilloscope bridge circuit amplifier [Fig. 1(b)]. The transducer was calibrated by measuring the height of the water column and calculating the resulting applied pressure. The computer was programmed to simultaneously record the applied hydrostatic pressure and to measure the corneal curvature.

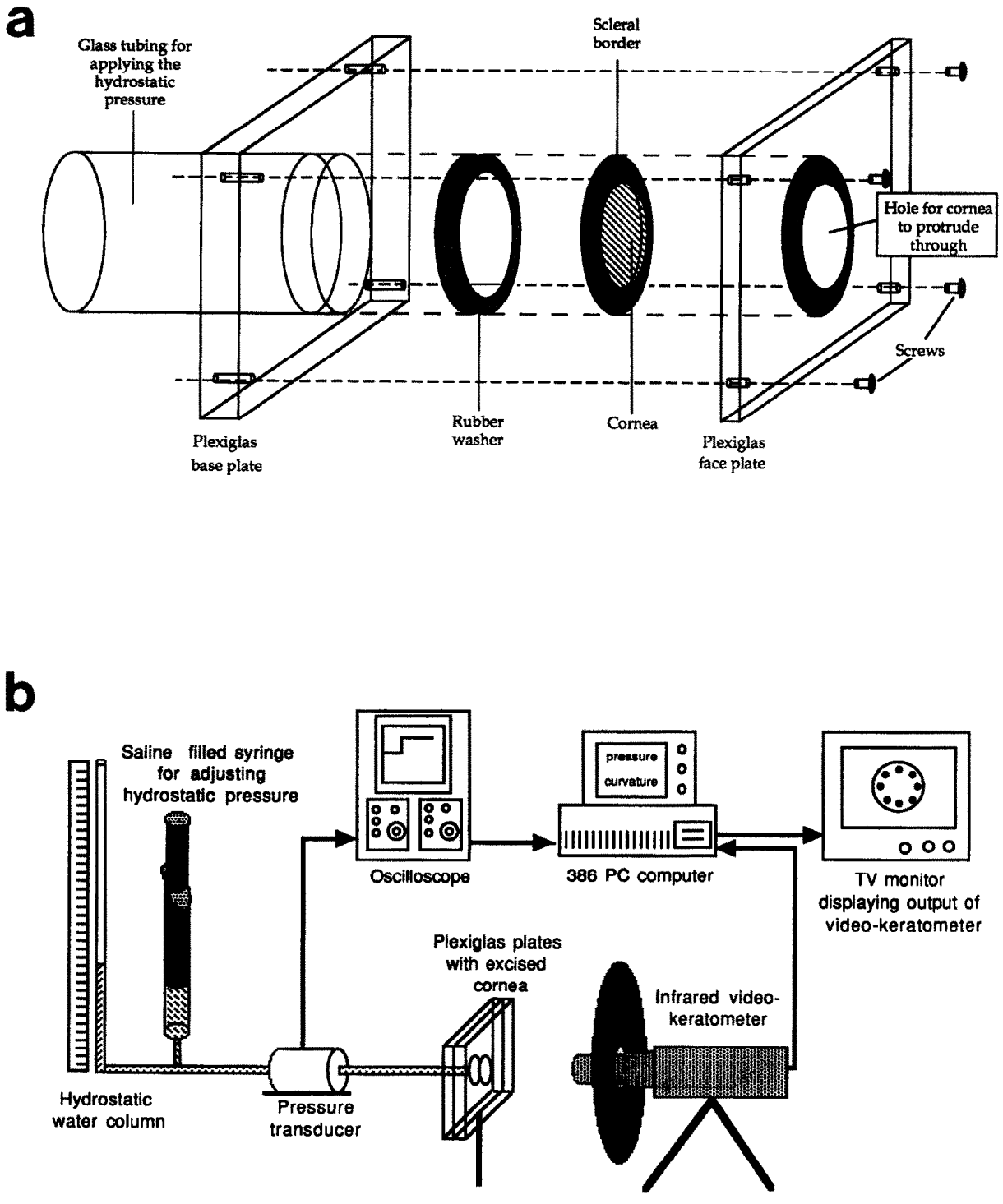

FIGURE 1. Diagram showing the apparatus used to measure changes in corneal curvature as a function of hydrostatic pressure applied behind excised corneas. (a) The excised cornea together with a few millimeters of surrounding sclera is clamped between two plexiglas plates. The hydrostatic pressure is applied behind the cornea through the glass tube, and the cornea protrudes through the hole in the face plate to allow the changes in curvature to be measured. (b) The plexiglas plate in (a) is positioned in front of a video-keratometer attached to a PC, which simultaneously measures the corneal curvature and the applied pressure behind the cornea. The applied pressure is adjusted by changing the height of the hydrostatic water column using the syringe. This setup allows for the rapid measurement of corneal curvature for a wide range of applied pressures.

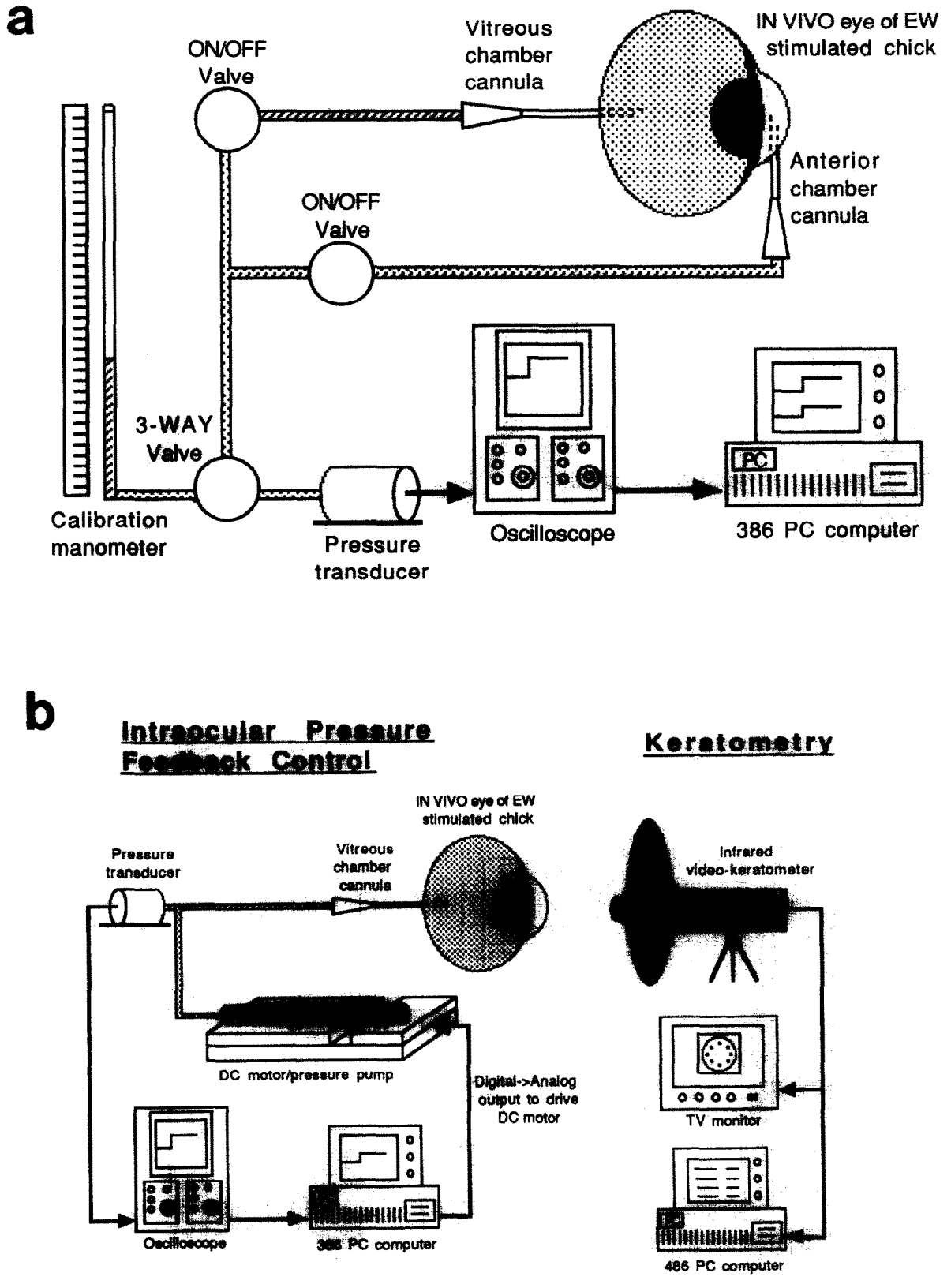

FIGURE 2. Diagrams of the setups for (a) measuring changes in IOP during EW-stimulated accommodation and (b) measuring changes in corneal curvature while modulating the IOP during EW-stimulated accommodation. (a) Chicks with electrodes implanted in the EW nucleus had their anterior and vitreous chambers cannulated. The cannulae were connected through a series of valves to a pressure transducer which was connected through an oscilloscope to a PC. The computer recorded changes in IOP during EW-stimulated accommodation. The pressure transducer was calibrated by measuring the height of water in the calibration manometer. (b) Chicks with electrodes implanted in the EW nucleus had their vitreous chambers cannulated. The cannula was connected to a pressure transducer and a pressure feedback pump. The pressure transducer was connected to a PC, which could simultaneously measure the pressure and drive the d.c. pressure pump. The IOP could be adjusted to a set level by infusing or removing saline from the eye. The changes in corneal curvature during EW-stimulated accommodation were measured using the video-keratometer for a range of set IOPs and accommodative amplitudes.

The rapidity of this technique ensured that the corneas of both eyes were used within 10 min of enucleation, thus minimizing possible artifacts due to edema or deterioration of the corneas.

Infrared video-keratometry

To measure the curvature of the cornea (either *in vitro* with the excised cornea protruding through the face plate or *in vivo* in the intact eye) the cornea was positioned in front of a video-keratometer as described previously (Schaeffel & Howland, 1987; Schaeffel, Glasser & Howland, 1988). Corneal curvature was expressed as dioptric power using the effective value of 1.362 for the refractive index of the cornea (Sivak, Bobier, & Levy, 1978). The video-keratometer was calibrated using a set of four steel ball bearings of known radius of curvature. The calibration curve demonstrated the precision of this technique to be better than 0.05 mm, which corresponds to approx. 0.9 D of corneal power in the chicken eyes used (mean corneal radius of curvature of 4.43 mm).

EW-stimulated accommodation

The EW nucleus is the dorsal subdivision of the oculomotor nuclear complex that, by way of the ciliary ganglion, provides parasympathetic innervation to the ciliary muscles of the eye (Reiner, Karten, Gamlin & Erichsen, 1983). Because electrical stimulation of the EW nucleus induces accommodation via the natural pathways (Troilo & Wallman, 1987) and results in accommodative changes most similar to those seen during natural accommodation (Schaeffel & Howland, 1987), we have used that technique here to more completely characterize the accommodative mechanism in chicks.

Eight 4-week old chicks (mean age of 31.6 days), while deeply anesthetized, had monopolar microelectrodes (SNEX100, 15 mm shaft length, 0.5 mm tip exposure, Rhodes Medical Instruments, Inc.) stereotaxically positioned at the coordinates of the right EW nucleus (0.9 mm anterior, 0.5 mm lateral and 3–4 mm dorsal of the zero position). The dorso–ventral position was adjusted as necessary to produce the strongest accommodative response as assessed by movements of the first Purkinje reflex on the corneal surface, by pupillary responses and from retinoscopy. Stimulating electrodes were then cemented to the skull using repair acrylic (Lang Dental Mfg. Co., Wheeling, Ill.). The chicks were removed from the stereotaxic unit and all experimental measurements were made while the chicks remained anesthetized.

Stimulus parameters. A mean resistance of 130 kΩ was measured across the stimulating electrodes in five chicks. Accommodation was stimulated with an S4G Grass stimulator (Grass Medical Instruments, Quincy,

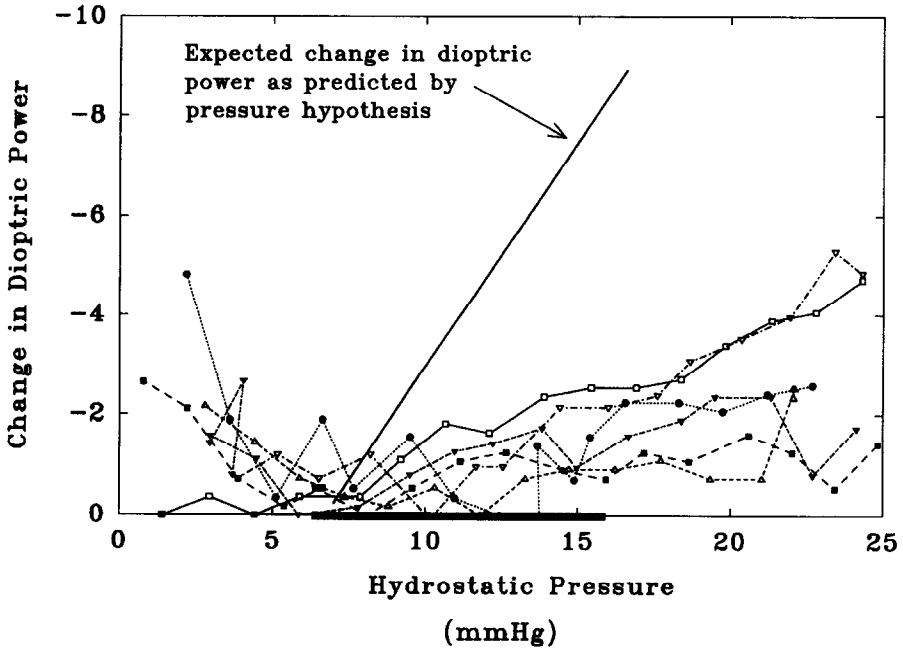

FIGURE 3. Changes in curvature (expressed in diopters) of six excised chick corneas as a function of hydrostatic pressure applied behind them. The change in dioptric power for each cornea is plotted relative to the steepest curvature recorded for that cornea (which did not necessarily occur at 0 mmHg). The thickened line along the abscissa (7–16 mmHg) represents the reported physiological range of hydrostatic pressures (Lauber *et al.*, 1970) over which the chick eye would theoretically be able to modulate IOP. Behavioral experiments have shown that chicks are capable of up to 9 D of corneal accommodation. The straight line represents the relationship between corneal power and hydrostatic pressure if the expected 9 D of corneal accommodation were mediated through changes in IOP (slope is 9 D/9 mmHg). The data for six corneas show that little more than a mean of 4 D of change in corneal power can be induced over 25 mmHg. There is a tendency for the corneas to collapse at 0 mmHg and to increase in curvature as the pressure behind them is raised towards 5 mmHg. From 5 to 25 mmHg, however, there is an overall tendency towards a *flattening* of the corneas with an increase in pressure (increasing negative values on the ordinate). A regression fit through the data for the six chicks can be represented by the regression equation $y = -0.081x - 0.475$.

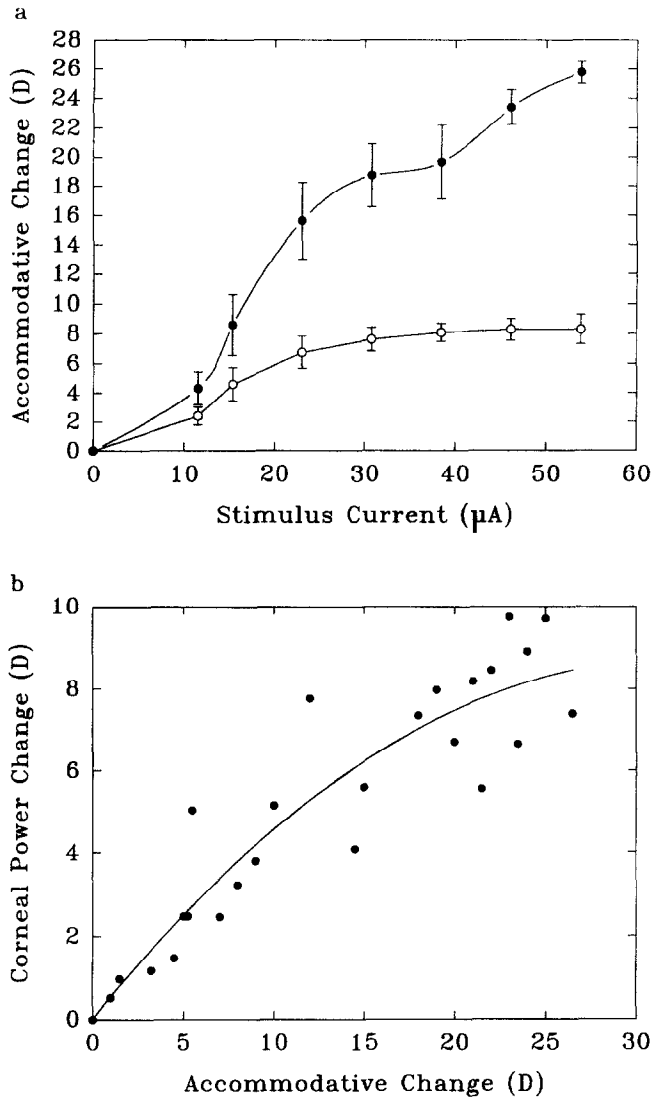

FIGURE 4. Graphs showing the relationship between corneal accommodation and the total accommodative response. (a) Graph of total accommodation (solid symbols) and corneal accommodation (open symbols) as a function of stimulus current for all EW-stimulated chicks. Stimuli of up to 55 μA produced a mean asymptotic total accommodative responses of up to 25.75 D ($\pm$SEM) and a mean asymptotic corneal accommodative response of up to 8.28 D ($\pm$SEM). (b) Graph of the relationship between change in corneal refracting power as a function of the total accommodative change. The dots represent data from eight EW-stimulated chicks in which total accommodation and corneal accommodation were measured as a function of stimulus current. The solid line represents a polynomial fit to the data ($y = 0.028 + 0.37x - 0.008x^2$; $R = 0.92$). From this line, we see that corneal accommodation shows an asymptotic relationship, therefore playing a relatively greater role at lower accommodative amplitudes. Corneal accommodation represents roughly 40% of the total accommodative amplitude with a maximum of 55% and a minimum of 32%.

Mass.) connected directly to the electrodes. Biphasic pulse stimuli were delivered at a frequency of 300 Hz, pulse duration of 4 msec and up to 10 V resulting in currents of up to 80 μA. Stimuli were maximized at a level that induced large accommodative changes without stimulating oculomotor movements. Averaged data from eight chicks indicate that the accommodative responses saturated at approx. 55 μA.

At the end of each experiment, lesions were made with a 5 V, d.c. stimulus for 5 sec to mark the location of the electrode tip in the EW nucleus. The electrode tip location was later verified histologically.

Neutralizing infrared photoretinoscopy. To measure the extent of the accommodative changes in the eye during EW stimulation, anesthetized EW-stimulated chicks ($n = 8$), with their right eyelids retracted, were refracted using infrared video-photoretinoscopy (Schaeffel, Farkas & Howland, 1987). Ophthalmic trial lenses were selected to best neutralize the retinoscopic reflex in the eye, a technique with a sensitivity of approx. 1 D. Chicks were refracted with the eye in the relaxed state and then for increasing stimulus currents up to 55 μA.

In vivo infrared video-keratometry. Corneal curvatures were measured in eight chicks using a video-keratometer as described previously. Anesthetized EW-stimulated chicks were positioned in front of the keratometer with their eyelids retracted to ensure that the keratometer light spots were centered within the pupil. Corneal curvatures were measured for each eye while in the relaxed state and again for a series of electrical stimuli applied to the EW nucleus. The change in corneal curvature was measured and expressed in dioptric power.

The video-keratometer was also used to demonstrate changes in the curvature of the peripheral cornea by viewing the eye off the optic axis in three cases. Anesthetized EW-stimulated chicks were positioned such that the light spots were reflected off the temporal margin of the cornea with the most temporal light spots being reflected from the corneo-scleral margin and the more central light spots reflected from the more central cornea. As with the on-axis keratometry, changes in the curvature of the cornea can be observed from movements of the reflected light spots. However, absolute changes in the curvature of the peripheral cornea could not be measured in this manner due to the aspherical nature of the peripheral cornea.

Slit-lamp observations. An ophthalmic slit-lamp (Bausch & Lomb) was used to observe accommodative changes in the eye during EW-stimulated accommodation. The slit-lamp provides an adjustable slit beam illumination that can be focused within the eye. The slit beam of light is observed with binocular objectives as it passes through the transparent optical media of the eye to provide an optical cross-section through the eye. Corneal thickness, anterior chamber depth, lens thickness and any changes that occur in the eye during accommodation can be observed in this way. Based on the known thickness of the cornea (250 μm), we estimate that we are able to see movements as small as 15 μm (approx. one-sixteenth the thickness of the cornea). The eyes of more than 10 anesthetized EW-stimulated chicks, with the lids retracted, were observed and videotaped using the slit-lamp.

IOP measurements

Following refraction and keratometry, anesthetized, EW-stimulated chicks were again immobilized in the stereotaxic unit and additional s.c. injections of 2%

lidocaine were administered around the orbit of the eye. Two cannulae (20 gauge syringe needles) were connected to a single flow-through blood pressure transducer (Radnoti, Glass Technology, Inc.) via a three-way valve system. After all air bubbles were removed from the fluid lines, one cannula was inserted into the posterior region of the vitreous chamber through the dorsal surface of the eye until it was visible through the pupil. The second cannula was introduced into the anterior chamber of the eye by inserting it s.c., dorsal of the auditory meatus and through the conjunctiva at the temporal corneo–scleral margin of the eye. The tip of the cannula was pushed through the cornea at the corneo–scleral border of the eye and into the center of the anterior chamber taking care to avoid the iris. This method of cannulating the anterior chamber ensured a good seal with no leakage

FIGURE 5. Photographs of keratometer light spots reflected off the corneal surface of EW-stimulated chicks. (a) Double exposure of the eye in the relaxed state (outer ring of light spots) and accommodated state (inner ring of light spots), demonstrating that the light spots reflected from the central cornea move concentrically inward as the eye accommodates. The movement of the light spots from the outer ring to the inner ring represents approx. 8 D of corneal accommodation. el, eye lid (with feathers attached); i, iris; n, nictitating membrane; p, pupil, s, sclera. (b, c) When viewed with the keratometer from the temporal edge of the eye, the light spots are reflected from the peripheral margin of the cornea. When the eye goes from the relaxed state (b) to the accommodated state (c), the light spots on the temporal edge are seen to move away from those on the more central cornea, representing a flattening of the cornea at the periphery. el, eye lids (upper and lower); i, iris; lr, lid retractor; n, nictitating membrane; p, pupil.

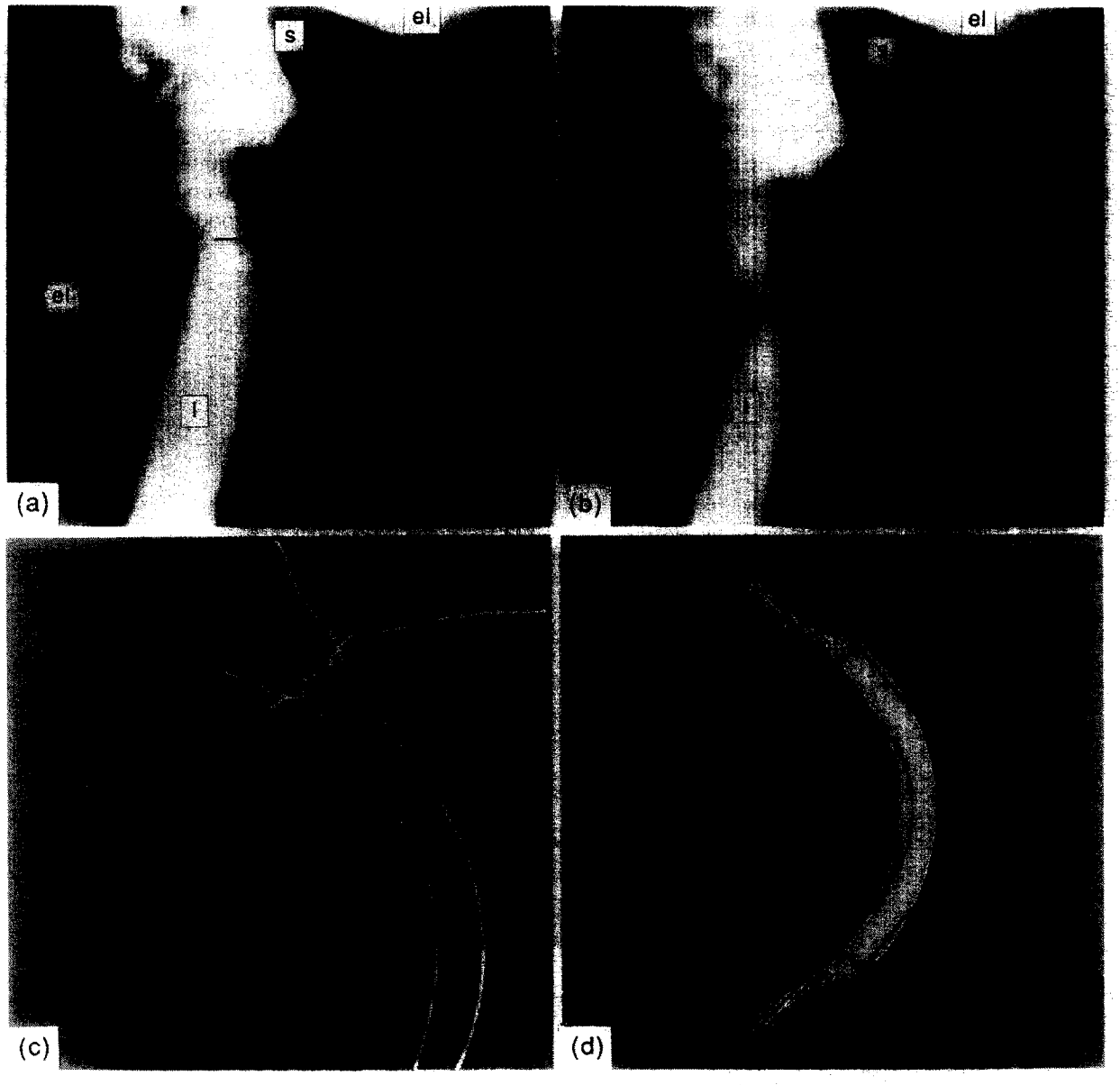

FIGURE 6. The mechanism of corneal accommodation. (a, b) Photographs of the superio–temporal corneo–scleral border of the right eye of an EW-stimulated chick in the (a) relaxed and (b) accommodated states under slit-lamp illumination. The eyelid (el—top and left) is held open by a lid retractor (giving it a squared appearance) to reveal the underlying sclera (s). The slit-lamp illuminates this region of the sclera and the associated cornea (c) showing the cross-sectional thickness of the cornea. Within the anterior chamber of the eye, the slit-lamp illuminates the pectinate ligament (between the small arrows) at the angle of the iris and also the anterior surface of the iris (i). The movement at the corneo–scleral border (large arrow), the flattening of the peripheral cornea, the stretching of the pectinate ligament, and the contraction of the peripheral musculature of the iris that accompany accommodation can be seen by comparing (a) and (b). (c) Tracings of the cornea and associated sclera as illuminated by the slit-lamp in the slit-lamp images [(a) and (b) above] in the relaxed state (white) and the accommodated state (black) show the relative changes from the relaxed to the accommodated state. Note: the movement of this peripheral region of the cornea is primarily into the eye during accommodation. Because the photographs in (a) and (b) do not represent a true cross-section of the cornea, the movement (from white to black) is largely *into* the page and not just from right to left as apparent from the tracings shown here. (d) The corneal accommodative mechanism is shown from a diagrammatic vertical cross-section through the eye. When the cornea goes from the relaxed state (white cornea) to the accommodated state (black outline), the ciliary muscle (striped area) contracts (in the direction of the straight arrows) to pull the inner lamella of the cornea at the corneo–scleral margin backward and under the corneo–scleral margin. The corneo–scleral margin is rotated inward (curved arrows) about the anterior apex of the scleral ossicles (black area), the peripheral cornea is flattened while the curvature of the central cornea is increased.

around the cannula and with negligible disruption of the cornea. The two cannulae were clamped in place to prevent any movement. Although the anterior chamber cannula may have resulted in a slight disruption of the corneal curvature, changes in corneal curvature, changes in curvature of the lens and normal pupillary changes were always observed during EW-stimulated accommodation.

Blockages of the cannulae occurred infrequently, but a T-tube at the base of each cannula allowed it to be cleared by inserting a fine wire through a rubber stopper in one end of the T-tube and into the cannula without

opening the pressure measuring system to the atmosphere.

The pressure transducer was connected via a bridge circuit amplifier oscilloscope through an analog-to-digital (A/D) converter to an IBM PC [Fig. 2(a)]. A d.c. equivalent of the stimulus trace was fed through a second input to the PC to enable simultaneous recording of the stimulus onset and duration. In this manner, a continuous recording of the pressure changes in the anterior chamber or vitreous chamber could be made together with the stimulus onset, offset and magnitude. The three-way valve system allowed the anterior chamber pressure, the vitreous chamber pressure, both chambers together, or a calibration manometer to be monitored at any one time by the pressure transducer.

Changes in IOP were also measured in four enucleated chick eyes maintained in Tyrode's solution in which accommodation was induced by extraorbital stimulation. Eyes were enucleated, the vitreous chamber cannulated, the IOP increased to 15 mmHg by saline infusion through the cannula, and the changes in IOP measured while stimulating the eye as described below. In this way, we were able to ensure that changes in IOP measured during EW stimulation are due to contractions of the intraocular muscles and not to artifacts of extraocular muscle contractions as seen in experiments on mammals (Jample & Mindel, 1967).

IOP regulation

In a second set of experiments designed to fully characterize the role of IOP in the corneal accommodative mechanism, a negative feedback pressure regulation system was developed through which the IOP could be set and maintained at any specified value [Fig. 2(b)]. A PC, which monitored the IOP through the pressure transducer as described above, was used to control a d.c. motor with a 3 cm^3 syringe attached to withdraw or infuse saline into the vitreous chamber of the eye of anesthetized EW-stimulated chicks ($n = 4$). For each set pressure value (from 5 to 40 mmHg in steps of 5 mmHg), accommodation was induced by stimulating the EW nucleus with stimuli of up to 55 μA and the resulting changes in corneal curvature were measured at each pressure/stimulus combination. The pressure feedback system had a response time of several hundred milliseconds and was able to fully compensate for minor pressure fluctuations within one second.

In vitro stimulated accommodation

To further study the accommodative mechanism, we electrically stimulated excised eyes maintained in oxygenated Tyrode's solution (Pilar & Tuttle, 1982). Ten 4-week old chick eyes were enucleated, cleared of all extraorbital tissues and glued to a plexiglas plate with cyanoacrylate glue. The plexiglas plate had a 12 mm bevelled hole drilled through it to allow the anterior scleral region to fit into the hole with the cornea protruding through the front of the plate. The plexiglas plate was firmly clamped and the eye could be observed, manipulated, or dissected while immersed in Tyrode's solution. The intraocular muscles were stimulated (100 V, 50 Hz, 15 msec duration, monophasic) using wire electrodes placed in solution. The eyes were dissected to allow direct observation of contractions of the ciliary and iris muscles and observations of corneal and lenticular movements. Eyes were found to remain viable for up to 2 hr under these conditions. We also used the slit-lamp to observe and videotape accommodative changes in more than five enucleated eyes during *in vitro* electrical stimulation. Although no attempt was made to measure the extent of accommodation in enucleated eyes, the accommodative changes observed are consistent with those seen during EW stimulation.

Eye morphology and histology

To examine the morphology of the anterior segment of the eye, eyes ($n > 10$) were enucleated and cleaned of extraocular muscles and connective tissue. Freshly enucleated, unfixed eyes were glued, using cyanoacrylate glue, at the equator of the globe into a bevelled hole drilled into a plexiglas plate. The plate was held immobilized in a well of a specially designed dissecting dish, immersed in Tyrode's solution, dissected, videotaped during electrical stimulation and photographed.

Eyes ($n = 6$) fixed in 4% glutaraldehyde fixative were blocked with horizontal or vertical cuts through the pupil. The blocks were dehydrated, cleared, embedded in plastic, serially sectioned at 5–10 μm and stained on a hot plate with toluidine blue or basic fuchsin/methylene blue for light microscopy.

RESULTS

Hydrostatic pressures applied behind excised corneas

One chick accommodating to hand held targets was shown to have up to 9 D of corneal accommodation. This result is in agreement with the behavioral measures of Schaeffel and Howland (1987).

Following enucleation and dissection, the curvatures of the excised corneas at physiological pressures (7–16 mmHg) were similar in magnitude to the curvatures measured in the intact eyes. Six chick eyes had a mean corneal curvature of 4.43 mm *in vivo* and the excised corneas a mean of 4.42 mm.

Over a wide range of applied hydrostatic pressures (0–25 mmHg), far exceeding the reported normal physiological range of 8–17 mmHg (Lauber, Boyd & Boyd, 1970), we were able to record changes in corneal curvature on the order of 3–4 D with a mean change of 3.76 D ($\pm$ 1.3 SD). The corneas tended to flatten as the applied hydrostatic pressures were increased. Over the physiological range of hydrostatic pressures, corneas flattened by 1.18 D ($\pm$ 0.62 SD). The corneas tended to collapse when no pressure was applied behind them, resulting in an initial increase in the curvature as the pressure was increased from 0 to 5 mmHg. This was followed by a gradual flattening of the corneas with further increasing pressure, as seen in Fig. 3.

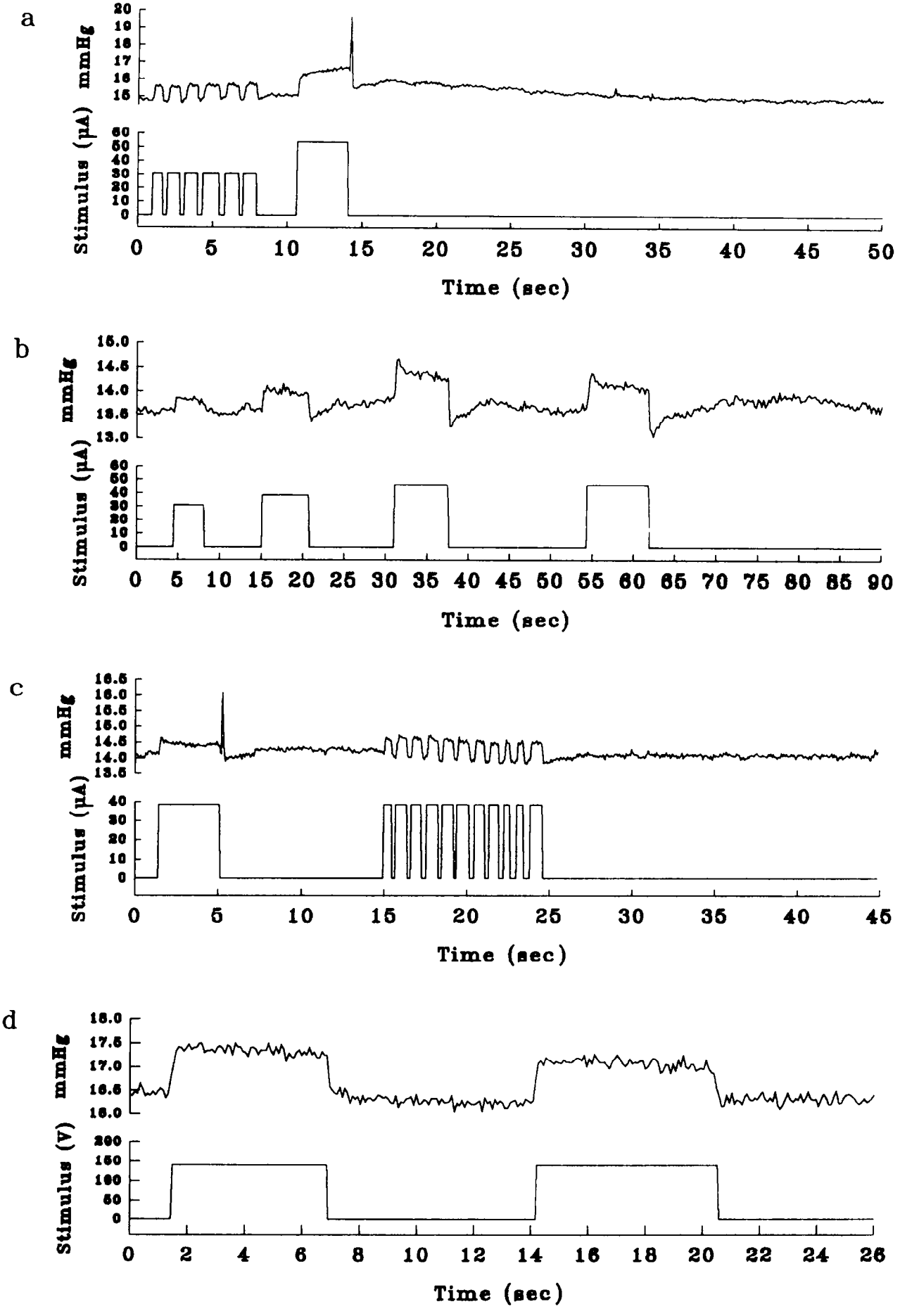

FIGURE 7. *Caption on facing page.*

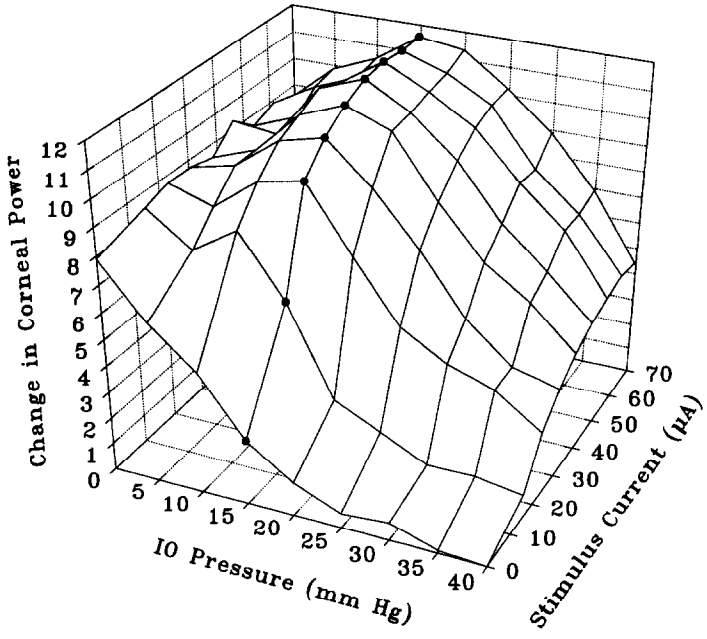

FIGURE 8. Example of change in corneal refracting power as a function of IOP and EW stimulation. Each data point represents the average of three separate measurements of corneal refracting power at the specific IOP and stimulus current. At normal IOP of 15 mmHg (solid symbols), the characteristic asymptotic relationship between corneal refracting power and stimulus current is seen with a maximal corneal accommodative response of about 9 D. Without any accommodation (current = 0), as the IOP is increased above normal IOP (15 mmHg), the curvature of the cornea is flattened, and as the IOP is decreased below normal there is an increase in corneal curvature toward the maximum. Note that in the *in vivo* condition pressure changes from 0 to 40 mmHg can result in up to an 8 D change in corneal power. At high IOP (40 mmHg) there is a maximum of only about 4.5 D of EW-stimulated corneal accommodation over the full range of stimulus currents. At 0 mmHg there is no change in corneal refracting power as a function of stimulus current.

EW-stimulated accommodation

For the eight EW-stimulated chicks used in this study, the electrode marking lesions were consistently located within the boundaries of the EW nucleus. Electrode placement was not always centrally positioned within the accommodative (lateral) subdivision of the EW nucleus, thus producing some variability in the accommodative range of individual animals.

Neutralizing infrared photoretinoscopy. Figure 4(a) (solid symbols) shows a plot of the mean total refractive change for all EW-stimulated chicks as a function of stimulus current as measured by neutralizing infrared photoretinoscopy. A mean maximal accommodative amplitude of 25.75 D was measured, showing a saturation of the accommodative response with stimulus currents of 55 μA and higher.

In vivo infrared video-keratometry. Figure 4(a) (open symbols) shows the range of corneal accommodation as recorded in eight chicks during EW-stimulated accommodation plotted as a function of stimulus current. A mean maximal change in corneal refracting power of up to 8.28 D was measured using infrared video-keratometry and as with the full accommodative response, an asymptotic relationship is observed between corneal curvature and stimulus current.

Figure 4(b) shows the relationship between changes in corneal curvature as a function of the total accommodative change. The quadratic polynomial fit shows an asymptotic relationship between corneal accommodation and total accommodative change reflecting a proportionally greater role played by the cornea at lower accommodative amplitudes. A similar relationship was reported by Troilo and Wallman (1987). Corneal accommodation accounts for a maximum of 55% of the accommodative change at the lower accommodative amplitudes and a minimum of about 32% at maximal accommodation. The average contribution to the total accommodative change is about 40%.

Changes in corneal curvature are readily observed as changes in the diameter of the ring of reflected

FIGURE 7 (opposite). Changes in IOP during accommodation. The upper trace of each pair shows the IOP recording, and the lower trace shows the accommodative stimulus. Note that the scales are different in each case. (a,b) Representative recordings from the anterior chamber of two chicks. The accommodative response is frequently followed by a blink of the nictitating membrane as seen by the spike in (a). Note that the magnitude of the increase in IOP from the blink is considerably greater than that resulting from EW stimulation. In (b) note also that an increased stimulus results in a greater increase in IOP. (c) Example of a recording from the vitreous chamber. The pressure changes recorded here are essentially identical to those in the anterior chamber. (d) Example of pressure changes recorded in an enucleated eye in which intraocular muscles were stimulated using bath applied electrodes. The IOP increases just as during EW-stimulated accommodation, indicating that pressure changes are due to contractions of the intraocular muscles only.

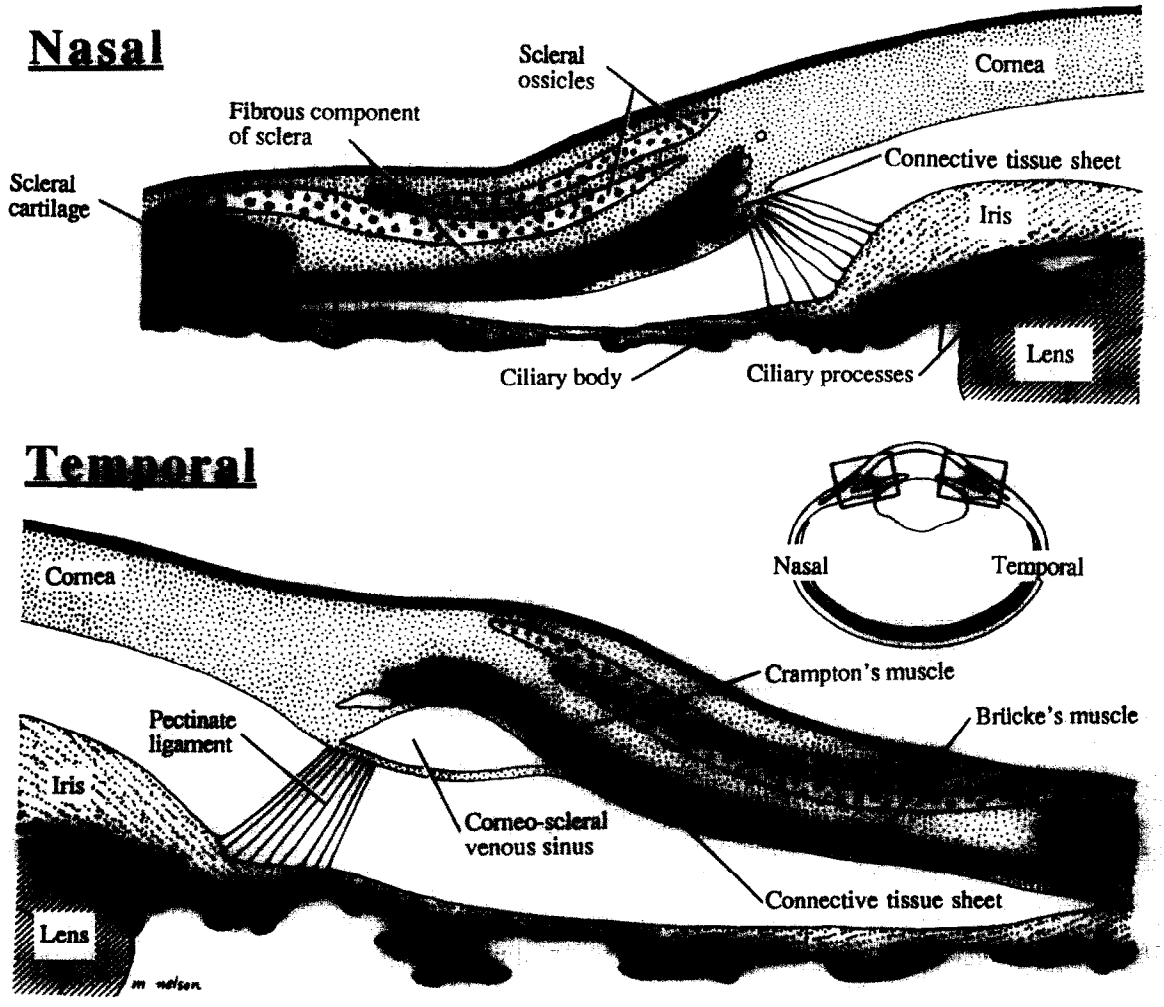

FIGURE 9. Diagrammatic representation of horizontal sections through the nasal and temporal regions of the anterior segment of the chick eye. The diagram represents sections taken midway through the pupil such that the radially oriented ciliary muscle fibers run parallel to the plane of section. Note the orientation of the fibers of the anterior ciliary muscle. Their relatively immovable origin is on the fibrous component of the sclera beneath the scleral ossicles. Their insertion is on the connective tissue sheet that is continuous with the corneal stroma. A contraction of this anterior ciliary muscle results in a pull on the inner lamella of the cornea, which causes an inward rotation of the peripheral cornea about the apex of the scleral ossicles. The curvature of the cornea in the unaccommodated eye would normally follow the curvature of the scleral ossicles similar to that depicted in the nasal section. A contraction of the ciliary muscle would result in changes in the curvature of the cornea similar to that seen in the temporal section, although here the flattening of the peripheral cornea is attributed to artifact. Those parts of the posterior muscle that are shown here also play a role in the corneal accommodative mechanism. These fibers connect between the connective tissue sheet continuous with the inner lamella of the cornea (in the temporal segment these compose the inner and outer walls of the corneo–scleral venous sinus) and the cartilage of the globe (not shown here, but see Fig. 11). During accommodation these fibers pull backwards on the connective tissue sheet that is continuous with the corneal stroma, so adding to the force on the inner lamella of the cornea. Inset is a horizontal section through a chick eye showing the boxed regions from which the two enlargements are drawn. Notice the marked naso–temporal asymmetry in the eye.

keratometer lights. In the central cornea, reflections of the ring of light spots move concentrically inward as the eye accommodates, indicating an increase in the curvature of the central cornea. Figure 5(a) shows a double exposure photograph of light spots reflected off the corneal surface of an eye in relaxed and accommodated states. The movement of the reflections from the outer ring to the inner ring corresponds to a change of approx. 8 D of corneal accommodation. If a similar demonstration is done with the lights reflected from the temporal cornea, the reflections from the temporal corneo–scleral margin move away from the reflections from the more central cornea [Fig. 5(b, c)], demonstrating that the curvature of the peripheral cornea flattens during accommodation.

Slit-lamp observations. The anterior and posterior surfaces of the cornea, the pectinate ligament and the connective tissue sheet that is continuous with the corneal stroma at the iridiocorneal angle are clearly visible under slit-lamp observation [Fig. 6(a, b)]. During accommodation, the ciliary muscle contracts pulling on the inner lamella of the cornea. The connective tissue sheet is visible as a white opaque tissue on the inner lamella of the cornea and is observed to be pulled

backward under the corneo–scleral margin when the eye accommodates. Accommodation is accompanied by a contraction of the iris sphincter muscle, which results in a stretching of the pectinate ligament against the inner lamella of the cornea at the iridiocorneal angle. This combined action causes an inward rotation of the corneo–scleral margin about the apex of the scleral ossicles [Fig. 6(c, d)], flattening the peripheral cornea and steepening the curvature of the central cornea.

IOP measurements

IOP was measured when the anterior chamber and vitreous chamber were cannulated in eight EW-stimulated chicks. The resting IOP varied widely in individual chicks from 9 to 34 mmHg, which may reflect individual differences or changes in IOP induced during cannulation of the eyes.

In all cases during EW-stimulated accommodation, relative increases in IOP were measured in the anterior and vitreous chambers. Figure 7(a, b) shows representative recordings of IOP measurements taken from the anterior chamber of two chicks, and Fig. 7(c) shows a representative recording taken from the vitreous chamber. In general, a stronger stimulus to the EW nucleus causes a greater increase in pressure [Fig.7(b)]. The overshoot in pressure at the onset of the stimulus and the undershoot after the stimulus terminates [Fig. 7(b)] are characteristics frequently observed.

Electrical stimulation of enucleated eyes maintained in Tyrode's solution causes increases in IOP similar in magnitude to those seen *in vivo* [Fig. 7(d)]. However, the *in vitro* responses differed in that the pressure did not always return completely to baseline when the stimulus was terminated, and the characteristic pressure overshoot and undershoot observed in the EW-stimulated chicks were absent.

IOP regulation

Figure 8 shows representative data from an experiment in which the IOP was regulated and the corneal curvature measured during EW-stimulated accommodation in four chicks. Each data point represents the average of three separate measurements of corneal curvature. The solid symbols represent changes in corneal curvature with EW stimulation at the resting IOP of the eye of this particular chick (15 mmHg). In the relaxed eye (stimulus = 0 μA), as the IOP is increased above 15 mmHg, the curvature of the cornea flattens (a decrease in corneal refracting power), and as the pressure is decreased, the corneal curvature increases towards its most curved condition (an increase in corneal refracting power). An 8 D change in corneal curvature is recorded as the pressure is increased from 0 to 40 mmHg. At a set pressure level of 15 mmHg (normal IOP for this chick), 8 D of corneal accommodation occurs with EW stimulation up to 55 μA. [This relationship is essentially identical to that seen in Fig. 4(a) (open symbols).] As the IOP increases above 15 mmHg the range of EW-stimulated corneal accommodation falls off until, at 40 mmHg, we see a minimum range of only about 4.5 D. A decrease in IOP to 0 mmHg results in the complete absence of EW-stimulated corneal accommodation.

Histology and morphology of the ciliary region

From our histology and dissections, we agree with previous observations (Suburo & Marcentoni, 1983; West, Sivak & Doughty, 1991), that the ciliary muscle of the chick eye is composed primarily of two morphologically distinguishable subdivisions based on their fiber orientations, origins and insertions. They are (i) the anterior division [as initially described by Crampton (1813)] and (ii) the posterior division [as initially described by Brücke (1846)]. Three distinct groups of muscle fibers with differing origins and insertions can be identified within the posterior ciliary muscle (Murphy & Glasser, 1993; Murphy, Glasser & Howland, 1994). From serial sections and from dissections, we find, contrary to Suburo and Marcentoni (1983) and West *et al.* (1991), that the anterior insertion of Crampton's muscle is at the inner lamella of the cornea as described by Beer (1893) and Goodge (1960) in other bird species. We also note that there is a marked nasal/temporal asymmetry of the eye in the horizontal plane (Figs 9 and 10).

The origin of Crampton's muscle is at the fibrous component of the sclera, medial to the scleral ossicles. In the nasal segment, Crampton's muscle inserts anteriorly to the inner lamella of the cornea and to the connective tissue sheet which is continuous with the stroma of the cornea (Figs 9 and 10). This connective tissue sheet is substantially more pronounced nasally, occupying the region in which the scleral venous sinus is found in the temporal quadrant. The scleral venous sinus is reduced nasally, as reported by Goodge (1960) and often appears only as several small channels (Fig. 10). In the temporal segment, Crampton's muscle inserts onto the inner lamella of the cornea and to the connective tissue sheet that makes up the outer wall of the corneo–scleral venous sinus. Serial sections and dissections of the temporal segment of the eye show a markedly trabeculated attachment of Crampton's muscle to the inner lamella of the cornea that is largely absent nasally (Fig. 10).

Brücke's muscle is actually composed of three distinct groups of muscle fibers distinguished by their differing origins and insertions (Murphy & Glasser, 1993; Murphy *et al.*, 1994). Of direct significance to the mechanism of corneal accommodation is only one group of fibers with no defined origin (fixed point) or insertion (movable point). These fibers attach between the connective tissue sheet continuous with the inner lamella of the cornea and the tenacular ligament [Figs 9 and 10 show the anterior portion of these fibers and Fig. 11(a, b) shows the posterior portion] as originally described by Müller (1857). The tenacular ligament [Fig. 11(b)] is an elastic fibrous tissue continuous with the *pars plana* of the ciliary body that connects the posterior end of Brücke's muscle to the cartilagineous sclera of the globe.

The whole ciliary muscle lies medial to the scleral ossicles with the anterior insertion of Crampton's muscle near the anterior tips of the ossicles. The pectinate ligament extends from the connective tissue associated with the anterior aspect of the outer wall of the scleral venous sinus to the angle of the iris, with a few strands attaching to the anterior iris.

DISCUSSION

Summary of results

(i) During accommodation, the central cornea steepens while the peripheral cornea is flattened.

(ii) This change in shape cannot be explained by changes in IOP as the pressure changes observed *in vivo*

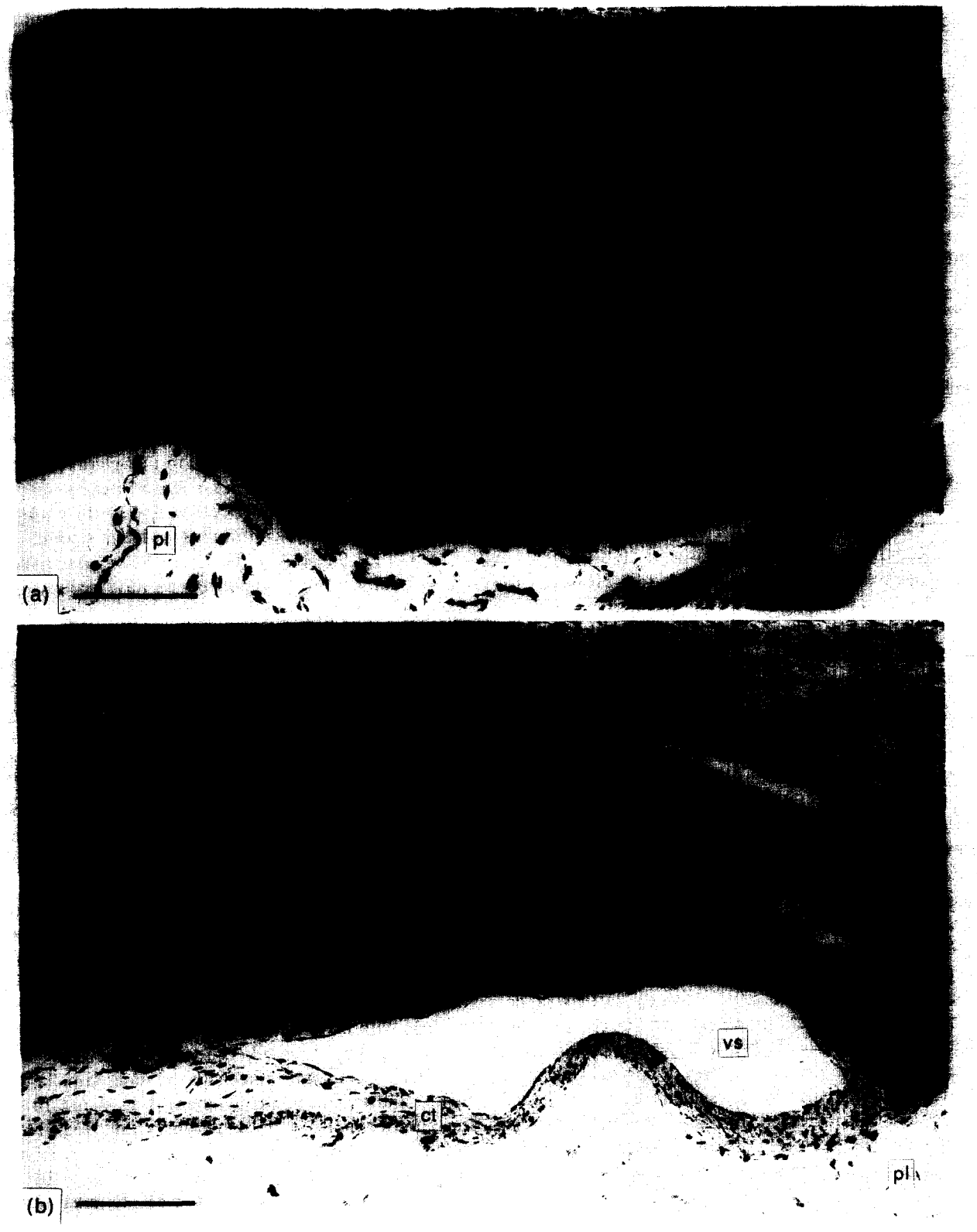

FIGURE 10. *Caption on facing page.*

during EW-stimulated accommodation are opposite to those that would be required to increase corneal curvature.

(iii) Corneal accommodation is accomplished by a contraction of the ciliary muscle that results in a pull on the inner lamella of the cornea.

(iv) Histological examination of the ciliary region of the eye supports this view.

Hydrostatic pressures applied behind excised corneas

Applying hydrostatic pressure behind excised corneas clamped at their scleral margins directly addresses the question of whether the elasticity of the avian cornea is sufficient to allow hydrostatic pressures to change the curvature. While the experimental conditions used result in an artificial rigidity at the scleral margin, we justified this constraint based on the natural rigidity imposed on the avian eye by the scleral ossicles. No movements of the sclera of the eye at the region of the ossicles was observed during accommodation in any of our *in vivo* or *in vitro* experiments. These experiments clearly demonstrate that, while more compliant than mammalian corneas, the avian cornea is insufficiently elastic to allow optically significant changes in corneal curvature. The results convincingly demonstrate that a pressure-mediated mechanism of corneal accommodation would require a decrease in IOP in order to increase the curvature of the cornea and would only be able to account for about 3 D of change in corneal power.

The pressure-mediated model of corneal accommodation suggested by Romano *et al.* (1989) assumed a decrease in pressure in the eye during accommodation as has been shown for mammalian eyes (Armaly, 1959). Our subsequent experimental evidence shows that there is an *increase* in IOP in the anterior chamber of the chick eye during accommodation, and this together with the requirement for a 9 D change in corneal power argues convincingly against a pressure-mediated mechanism of corneal accommodation.

EW-stimulated accommodation

Our results from EW-stimulated accommodation demonstrate that up to 25.75 D of accommodation are accompanied by changes in corneal curvature, representing changes in corneal refracting power of up to 8.25 D. This agrees well with our behavioral measurements of the range of corneal accommodation and with one other published report (Schaeffel & Howland, 1987). Troilo and Wallman (1987), however, found only 3.9 and 6.1 D of corneal accommodation from EW-stimulation and nicotine sulfate stimulation respectively with a maximum accommodative amplitude of 15 D. Although this most likely represents a less than maximal accommodative response, our results agree with Troilo and Wallman (1987) in that corneal accommodation represents approx. 40% of the accommodative ability of chick eyes and that it plays a relatively greater role at lower accommodative amplitudes.

Changes in IOP

Our observations of increases in IOP during electrical stimulation of excised avian eyes agree with previous results (Hess, 1909) and support the hypothesis that an increase in IOP during EW stimulation is not an extraocular muscle-mediated effect, as reported in mammalian studies (Jampel & Mindel, 1967). From our observed changes in IOP during accommodation, we can provide several conclusive reasons why corneal accommodation is not pressure-mediated: (i) increasing the pressure in the eye flattens the curvature of the cornea; (ii) the greatest curvature of the cornea obtained by reducing the IOP to 0 mmHg is still less than the maximum curvature attained during accommodation; (iii) maximum increases in IOP recorded during EW-stimulated accommodation (approx. 5 mmHg) are insufficient to induce significant changes in corneal curvature. (That a blink of the nictitating membrane across the cornea, a muscle action not involved in accommodation, results in

FIGURE 10 (*opposite*). Horizontal sections through the (a) nasal and (b) temporal anterior segment of the left eye of a 4-week old chick showing the insertion of the anterior ciliary muscle to the inner lamella of the cornea. The nasal–temporal asymmetry seen between (a) and (b) is characteristic of this plane of section. (a) The origin of the anterior ciliary muscle (cm) fibers is to the fibrous component of the sclera (fs) beneath the scleral ossicles (so). The insertion of these fibers is to the inner lamella of the cornea on the corneal stroma (cs) and to the connective tissue sheet (ct) that is continuous with the corneal stroma. This connective tissue sheet courses backward in the eye receiving the insertions of the anterior ciliary muscle fibers and more posteriorly the anterior insertion of the posterior ciliary muscle fibers around the nerve (n). Two to three such fibers of the posterior ciliary muscle can be seen in this section. The fibers of the pectinate ligament (pl) and a portion of the ciliary body (cb) can be seen in this section. Note the almost complete absence of the corneo–scleral venous sinus in the nasal segment. The two small openings near the anterior insertion of the ciliary muscle are all that can be seen of this sinus in the nasal segment. (b) In the temporal segment of the eye the origin of the anterior ciliary muscle (cm) is to the fibrous component of the sclera (fs) beneath the scleral ossicles (so). The anterior insertion of the ciliary muscle is to the inner lamella of the cornea and on the outer wall of the corneo-scleral venous sinus (vs) that is composed of a connective tissue sheet (ct) continuous with the corneal stroma (cs). In the temporal quadrant of the eye the corneo–scleral venous sinus is markedly enlarged and often tri-lobed as seen here. The openings of the sinus can be distinguished from blood vessles (bv) by the absence of circular muscle fibers surrounding them. The multi-lobed sinus gives the ciliary muscle a trabeculated attachment to the inner lamella of the cornea (at the label cs). It is through these trabeculations that the force of contraction is exerted on the inner lamella of the cornea. The connective tissue sheet that makes up the outer wall of the sinus courses backward in the eye and provides the anterior insertion point of fibers of the posterior ciliary muscle [see Fig. 11(b)]. Note that the insertion of the ciliary muscle to the inner lamella of the cornea is roughly 0.5 mm anterior of the apex of scleral ossicle in the temporal segment, whereas this is not the case in the nasal segment. Methacrylate embedded, 10 μm thick sections, stained with basic fuchsin and methylene blue. (Scale bar = 0.1 mm.)

a greater increase in IOP than does the act of accommodation further argues that changes in IOP are a consequence of the accommodative mechanism rather than a cause of it.)

Our IOP regulation experiments provide a plausible explanation of why Sivak *et al.* (1986) reported the absence of corneal accommodation from electrically stimulated chick eyes *in vitro* in which an opening had been made in the back of the globe. As suggested by Troilo and Wallman (1987) and illustrated in Fig. 8, there is a requirement of a normal positive IOP of 15–20 mmHg in order for corneal accommodation to occur. A further observation from this experiment is that, in increasing the absolute value of IOP from 0 to 40 mmHg *in vivo*, an 8 D decrease in corneal power occurs. While this is considerably more than that

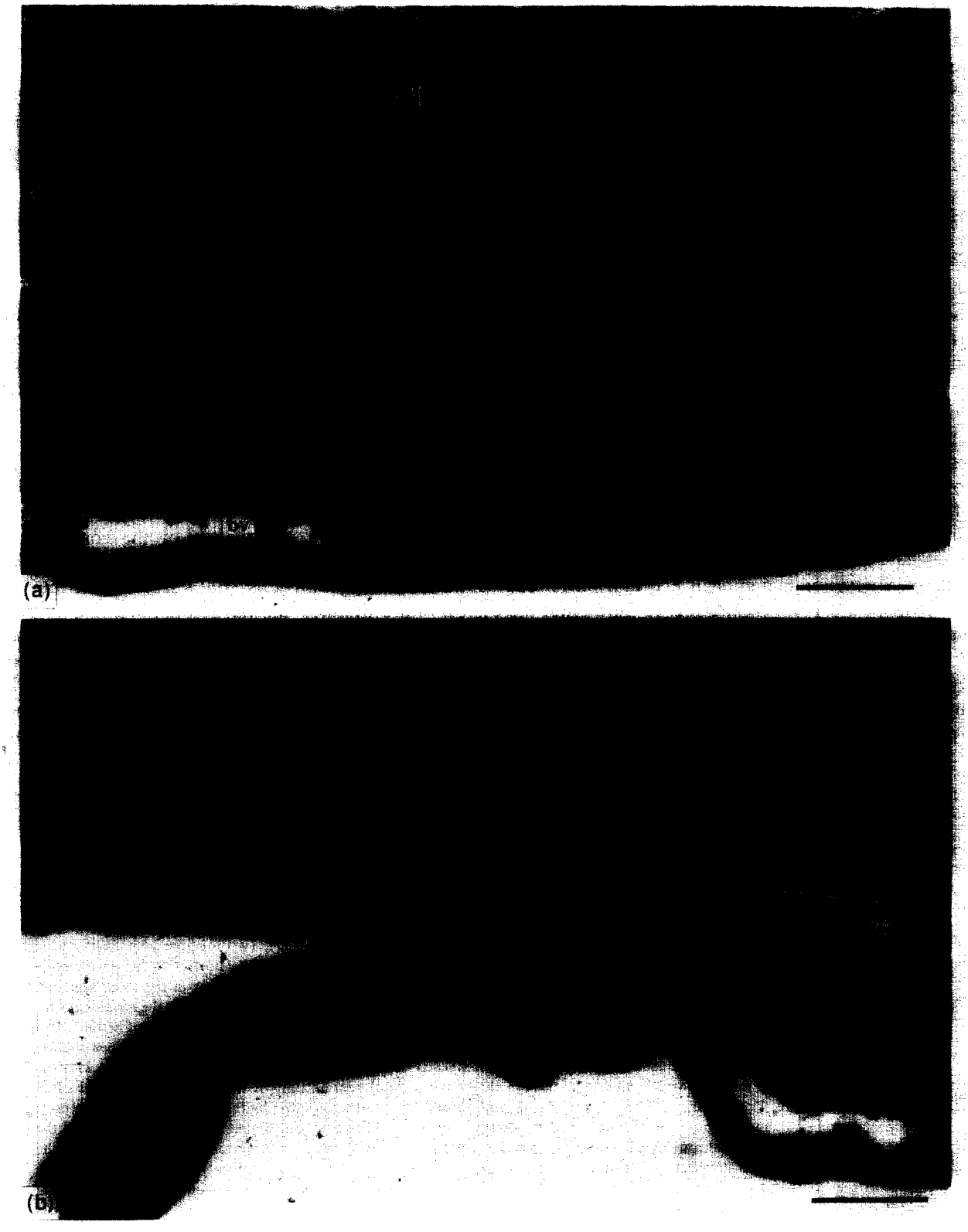

FIGURE 11. *Caption on facing page.*

measured in the *in vitro* experiments, the trend towards corneal flattening with increasing pressure is the same. The greater change in corneal curvature recorded in the *in vivo* condition possibly reflects some degree of flexibility in the sclera that has been restricted through clamping the corneo–scleral margin in the *in vitro* experiments.

The role of the scleral ossicles

Our experiments do not directly address the role that the scleral ossicles play in accommodation, but the origin of the ciliary muscles to the fibrous sclera adjacent to the ossicles, the inward rotation of the cornea about the anterior apices of the ossicles and the location of the ciliary muscles adjacent to the ossicles suggests that they serve an important function in accommodation of the avian eye. Accommodation is accompanied by a contraction of the iris sphincter muscle which stretches the pectinate ligament at the iridiocorneal angle, further assisting the inward rotation of the peripheral cornea. This together with a contraction of the ciliary muscles must result in a considerable inward force applied to the limbal region of the eye. Although the scleral ossicles of the chick eye do not constitute a completely rigid structure but have some flexibility by virtue of being composed of 14 individual overlapping plates, we were unable to observe and do not believe that the tips of the ossicles are pulled inward as part of the accommodative mechanism as described by Slonaker (1918). It is more likely that the scleral ossicles provide substantial structural reinforcement to the sclera against the force of contraction of the muscles of accommodation as well as the anterior apex of the ossicles acting as a fixed pivot point about which the corneo–scleral margin can rotate.

The source of the increase in IOP

Our experiments do not allow us to identify the precise source of the rise in IOP during accommodation, but our observations have identified several possible components. Flattening the peripheral cornea in order to steepen the central cornea is consistent with a reduction in the area under the cornea (Howland & Rand, unpublished observations) which would act to increase the IOP. We believe the major component of the pressure change is due to a contraction of Brücke's muscle against the tenacular ligament. Brücke (1846) described this muscle as the "tensor choroideae" and attributed its function to tensing the choroid with the included retina and vitreous body. We have observed that the tenacular ligament is stretched during electrical stimulation of dissected eyes *in vitro*. It is clear that the entire ciliary body and the *ora serrata* of the retina are pulled forward towards the posterior lens surface. In the intact eye this would act to pull the retina and choroid forward at the region of the *ora serrata* and so act to pull the vitreous towards the posterior surface of the lens as described by Brücke. This action most likely represents an aspect of the lenticular accommodative process, possibly providing a force to the posterior lens surface during accommodation (Brücke, 1846; Glasser, Murphy, Troilo & Howland, 1994). We cannot completely discount the possibility that there is a decrease in the equatorial diameter of the eye that is due to a buckling of the sclera in the region of the scleral ossicles as suggested by Slonaker (1918) for the eye of the English sparrow. Given that we have never observed such movements of this region of the chick eye during accommodation this seems unlikely.

The mechanism of corneal accommodation

In sharp contrast to some previous reports (Suburo & Marcentoni, 1983; West *et al.*, 1991), we report that a direct ciliary muscle-mediated mechanism of corneal accommodation in chicks is entirely consistent with the morphology of the ciliary accommodative apparatus. When the ciliary muscles contract, there is an inward and backward pull exerted on the inner lamella of the cornea by the anterior insertion of Crampton's muscle. This, together with increased tension on the pectinate ligament, results in an inward rotation of the cornea at the corneo–scleral margin about the anterior apex of the scleral ossicles, flattening the peripheral cornea and increasing the curvature of the central cornea. These changes are

FIGURE 11 (*opposite*). Horizontal sections through the temporal anterior segment of the left eye of a 4-week old chick showing (a) the posterior ciliary muscle and (b) the tenacular ligament. (a) Section showing the fibers of Brücke's muscle [the posterior ciliary muscle (cm)] which are located posterior to Crampton's muscle. The origin of the most posterior fibers of Crampton's muscle to the fibrous component of the sclera (fs) can be seen above and to the right of the nerve (n). These fibers course forward to insert to the inner lamella of the cornea [Fig. 10(b)]. The fibers of Brücke's muscle are located to the left of as well as below the nerve. Two distinct divisions can be seen: (i) the fibers located above and to the left of the nerve originate on the fibrous component of the sclera below the scleral ossicles (so) and course posteriorly in the eye to insert on the tenacular ligament (plate b). It is unlikely that this group of fibers plays any role in corneal accommodation, but they probably play a role in lenticular accommodation and in the rise in IOP during accommodation (see Discussion). (ii) the fibers located below the nerve extend between the connective tissue sheet continuous with the corneal stroma [Figs 9 and 10(b)] and the tenacular ligament (b). When these fibers contract, they pull on the inner lamella of the cornea through the connective tissue sheet. The ciliary body (cb) with a large partially blood filled blood vessel (bv) can be seen below the muscle fibers. The anterior apex of the scleral cartilage (sc) can also be seen in this section. (b) Section showing the tenacular ligament (tl), the elastic fibrous tissue that connects the posterior ciliary muscle (cm) with the cartilagineous sclera (sc) of the globe. Three fibers of the posterior attachment of Brücke's muscle to the tenacular ligament can be seen at the extreme right hand edge of the plate (at the label cm). The tenacular ligament courses backward in the eye to terminate under the scleral cartilage. The *pars plana* of the ciliary body (cb) at the region of the *ora serrata* is connected to the cartilage by the tenacular ligament at this point. The fibrous layer of the ciliary body becomes vascularized to compose the choroid just posterior to this point. When the ciliary muscle contracts, the tenacular ligament is stretched, pulling the ciliary body forwards. The elasticity of the tenacular ligament returns it to its rest position when the ciliary muscle is relaxed. The fibrous component of the sclera (fs) can be seen beneath the scleral cartilage. (Scale bar = 0.1 mm.)

consistent with: (i) the movements seen during accommodation at the corneo–scleral margin of the cornea; (ii) the histology and morphology of the eye; and (iii) the original hypothesis of Beer (1893) based on his observations of several other bird species a century ago.

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