**Table IV.** Inhibition of human tear breakdown of Hip-His-Leu

| Agent | Concentration | Enzymatic activity (nmol/min/ml) | | |
|---|---|---|---|---|
| | | Control | With inhibitor | % inhibition |
| EDTA | $8 \times 10^{-5}$ M | 14.77 | 1.08 | 93 |
| Bradykinin | $3 \times 10^{-5}$ M | 12.19 | 0.25 | 98 |
| Captopril | $5 \times 10^{-4}$ M | 4.23 | 0 | 100 |
| | $5 \times 10^{-5}$ M | 4.23 | 0 | 100 |
| | $5 \times 10^{-6}$ M | 4.23 | 0.03 | 99 |
| | $5 \times 10^{-7}$ M | 4.23 | 0.43 | 90 |

ever, other differences in ocular physiology have been shown to correlate with eye color. Millodot[9] found that blue-eyed people had greater corneal sensitivity than brown-eyed people, and Lee and Robinson[10] found that the rate of corneal metabolism of pilocarpine was at least 2 orders of magnitude greater in pigmented than in albino rabbits.

In this paper we have reported the presence of an enzyme activity in ocular fluids capable of catalyzing the formation of angiotensin II. These observations and those of other investigators showing ACE activity in ocular tissues[4] suggest that there may be a physiological function for this octapeptide in the eye.

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**Key words:** angiotensin converting enzyme, angiotensin, bradykinin, tears, aqueous humor

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## Naturally occurring strabismus in monkeys (*Macaca nemestrina*).

LYNNE KIORPES AND RONALD G. BOOTHE.

*Seven naturally strabismic monkeys* (Macaca nemestrina) *were identified. Five of these monkeys were examined by ophthalmologists. No ophthalmoscopically obvious cause for the squint was found in any case. Of those five animals, two were tested behaviorally on visual responsiveness and visual acuity. The acuity of both eyes of both monkeys was somewhat poorer than normal. In addition, an amblyopia of 0.8 octaves was found for one monkey and 0.6 octaves for the other. The existence of naturally strabismic monkeys supports the utility of the macaque as an animal model for studying strabismus and amblyopia.*

Clinical data have established that there is a close relationship between strabismus and amblyopia in humans.[1, 2] Strabismus sometimes develops secondarily to amblyopia as in cases of organic visual impairment. On the other hand, amblyopia often results from the presence of strabismus. There are questions about the development of strabismus and amblyopia that have not been answered by clinical studies. Some of these questions, e.g., those concerning the physiological or neuroanatomical bases for strabismus and ambly-

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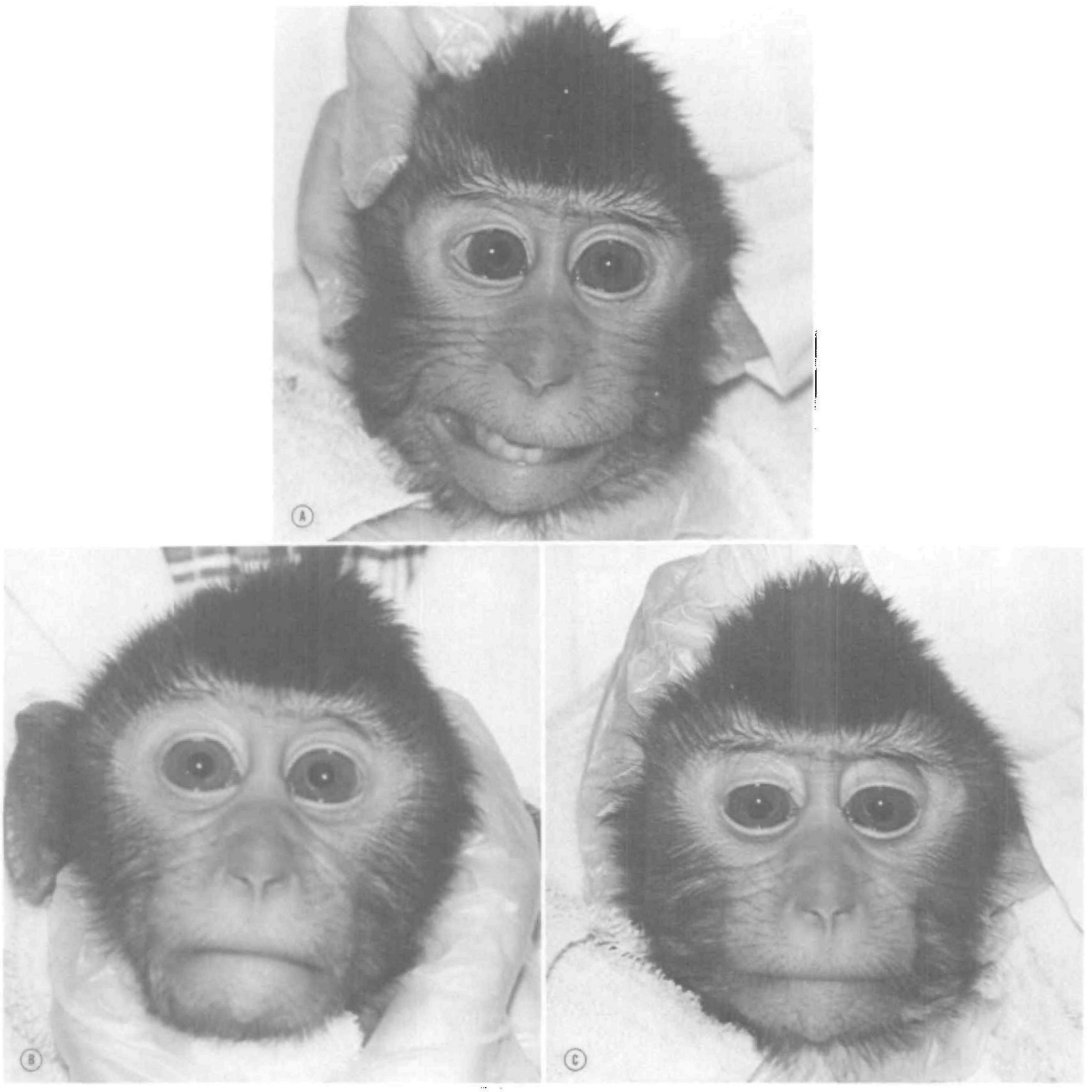

Fig. 1. Representative photographs of monkey KAY at 30 weeks, with the corneal reflex showing the nature of the squint.

opia, may be more readily investigated in an animal model.

Von Noorden and Dowling[3] have attempted to establish the macaque monkey as an animal model for studying human strabismus and amblyopia. They demonstrated that surgical esotropia created during the first 12 weeks after birth in infant macaque monkeys results in an amblyopia. But surgical exotropia does not result in an amblyopia, nor does esotropia if it is produced later than 12 weeks postnatally.

All these results are consistent with clinical findings for humans with natural strabismus. This suggests that the macaque monkey is indeed a good animal model. However, the adequacy of this model has been challenged on two major grounds.[4] First, surgical strabismus is always incomitant, whereas the typical infant human clinical case is comitant. Incomitant squints do occur though, and although they are not common, the macaque response to the condition of incomitant esotropia is similar to that of the human.

Second, Jampolsky[4] has argued that the macaque oculomotor system is different from that of the human, in part because monkeys do not naturally develop strabismus. This suggests that in

**Table I.** Ophthalmologic assessments

| Monkey | Age (weeks) | Refractive error | | Fundus | |
|---|---|---|---|---|---|
| | | OD | OS | OD | OS |
| KAY | 35 | +4.00 S | +2.50 S | Normal | Normal |
| | 45 | +3.00+1.00×90 | +2.00+.50×90 | — | — |
| | 53 | +4.00+0.50×90 | +5.00 S | Normal | Normal |
| ANU | 12 | +3.00 S | +4.00+0.50×90 | Normal | Normal |
| | 20 | 0.00+0.75×180 | −0.25+0.50×180 | Normal* | Normal |
| JAX | 10 | +2.75+0.25×70 | +2.75+0.25×110 | Normal | Normal |
| SAY | 39 | +8.00s | +8.00s | Normal | Normal |
| MAA | 5 yr | +6.50+0.50×180 | +6.00s | Normal | Normal |

*Slight decrease in pigmentation above and below optic nerve head.

**Table II.** Behavioral assessment of vision

| | Informal tests | | | | | | Acuity | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Age (wk) | Pupillary reflex | Following object | Guided reaching | Avoidance | OKN | Age (wk) | Procedure | OD (min arc) | OS (min arc) |
| KAY | 21 | + | + | + | + | + | 19 | FPL | 7.23 | 7.75 |
| | | | | | | | 42 | Operant | 8.75 | 6.20 |
| | | | | | | | 64 | Operant | 7.47 | 4.86 |
| ANU | 1 | NT | − | − | − | NT | 7.5 | FPL | 10.3 | 10.9 |
| | 3 | + | + | + | + | NT | 11 | FPL | 9.8 | 6.7 |
| | 12 | + | + | + | + | − | | | | |

+ = function present; − = function absent; NT = function not tested.

spite of the demonstrated structural[5] and functional[6] similarity between the human and macaque oculomotor systems, the monkey system must possess a unique property responsible for keeping the eyes straight.

We now have evidence that macaque monkeys do naturally develop strabismus. Over the past year, we have observed seven macaque monkeys with some form of natural strabismus. The kinds of strabismus seen are similar to those found in human patients. We have examined five of these monkeys and found no ophthalmoscopically obvious cause for the deviation in any case. These cases support the utility of the macaque monkey as a model for studying human strabismus and amblyopia.

***Methods.*** The monkeys described in the present report are pigtail macaques *(Macaca nemestrina).* We have conducted extensive testing with two cases, monkeys KAY and ANU. They were born without complication, in the Infant Primate Facility at the University of Washington, separated from their mothers on or before 3 days after birth, and reared thereafter according to normal infant laboratory protocol.[7] As part of the normal protocol, all infant monkeys were observed in their home cages and/or in a playgroup situation daily. During these observation periods, data were collected on each animal's behavior, and any unusual behaviors or general abnormalities were noted. It was during such routine observation periods that both KAY and ANU were first noted to have strabismus.

Subsequent to identifying KAY and ANU, we conducted a general screening of the colony. The colony includes the Regional Primate Research Facility in Seattle, Wash. (Infant Primate Facility), and Medical Lake, Wash. (Field Station and breeding colony). The screening was primarily observation of animals in their home cages. The observer sat quietly outside the cages until the animals adapted somewhat to his/her presence. Eye alignment was then observed in each animal as it moved about the cage. Five additional animals were identified during the course of the screening.

All clinical evaluations were performed by one of three ophthalmologists. The refractions and fundus examinations were done under cycloplegia, either 1% cyclopentolate (Cyclogyl; Alcon Laboratories, Inc., Fort Worth, Texas) or 1% atropine while the monkeys were lightly anesthetized with ketamine hydrochloride (Vetalar; Parke, Davis & Co., Detroit, Mich.).

Documentation of the squints was accomplished

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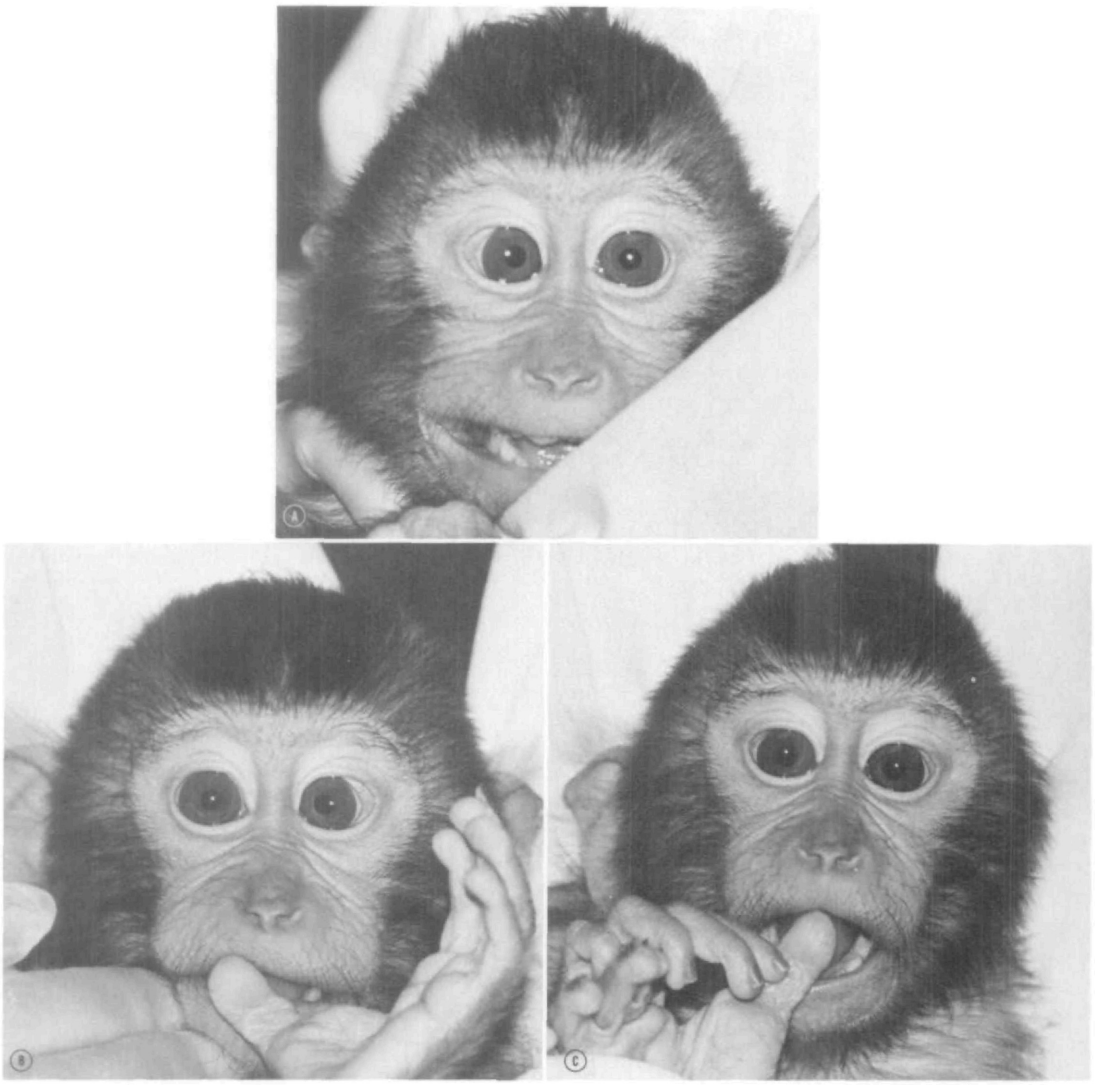

Fig. 2. Representative photographs of monkey ANU at 20 weeks, with the corneal reflex showing the nature of the squint.

with corneal reflex photography (the Hirschberg test).[1, 2] The corneal reflex method is a relatively inaccurate means of measuring the angle of squint; thus we used the photographs primarily for documentation purposes. Other information concerning the status of the squints was obtained by observation or cover test.[1, 2] We performed primarily the cover-uncover test using an apple slice as a target. The test was done at 0.3 and 1 meter. It was extremely difficult to keep the older animals' attention for more extensive cover testing, since they actively avoid anything in front of their eyes.

Behavioral evaluations were of two types: visual responsiveness and visual acuity. The informal tests of visual responsiveness have been described in detail previously.[8] Visual acuity assessments were accomplished either by the Teller forced-choice preferential looking method (FPL) or by operant methods, both of which have been described in detail previously (see ref. 9).

***Results.*** Seven naturally strabismic monkeys have been identified. Five of these monkeys were examined ophthalmoscopically. Two were also assessed behaviorally. The other five cases have not

been examined as extensively and are listed with the squints described as determined by observation. Available ophthalmoscopic data are presented in Table I.

*Case 1.* Monkey KAY's strabismus was first noted at 15 weeks of age. A series of photographs showing the esotropia with the corneal reflex are presented in Fig. 1, *a* to *c*.

These photographs are representative of the general nature of the squint. The deviation is larger when KAY fixates with her left eye (Fig. 1, *a*) than with her right eye (Fig. 1, *b*). Her deviation is larger at near than at far distance and unapparent when she is not fixating (Fig. 1, *c*). She alternates fixation but prefers her left eye. Ophthalmologic examination revealed her left eye to be her least hyperopic eye at the time the photographs were taken, 35 weeks (see Table I). The refraction has apparently changed since then (e.g., 53 weeks in Table I) but the nature of the squint has not. Note that the fundi were examined twice and in both cases were found to be normal.

Informal tests of KAY's visual responsiveness were unremarkable. She performed all tested functions with each eye when assessed at 21 weeks (Table II). Acuity was somewhat remarkable, however. When assessed at 19 weeks by FPL, acuity was similar for her two eyes, approximately 6/45 Snellen, but was poorer than normal. Unpublished data from our laboratory show normal monocular visual acuity for monkeys that old to be 2 to 4 min arc, or 6/12 to 6/24 Snellen. Six months later when she was tested with operant procedures, slight improvement was found in acuity; however, there was a consistent difference between her eyes. The interocular acuity difference was about 0.5 octaves* in the direction predicted on the basis of the initial anisometropia (Table I). Subsequent testing at 14 months confirmed the existence of an amblyopia (0.8 octaves).

*Case 2.* Monkey ANU's strabismus was first noted at 4 weeks of age. He was noted to have abnormal eye movements at 11 days, but the eyes did not begin to cross until 25 days. The series of photographs in Fig. 2 are representative of ANU's comitant esotropia. The amount of deviation is about the same whether he is fixating with his left eye (Fig. 2, *a*) or his right eye (Fig. 2, *b*) or is apparently looking with both eyes (Fig. 2, *c*). In addition, the deviation is approximately the same for near and far distance (by observation). He alternates and as yet shows no clear preference.

Ophthalmologic examination of ANU (Table I) at 12 weeks showed a common degree of hyperopia for a young monkey, with the right eye being slightly less hyperopic than the left. Fundi were both found to be normal. A second examination at 20 weeks revealed a rather large change in refractive error but little difference between the eyes. Fundus examination at this time was somewhat remarkable in that there was slightly decreased pigmentation above and below the optic nerve head in the right eye. According to the examining ophthalmologist, this finding is a common variation that is of no particular significance when seen in a human. The fundus of the left eye was unremarkable.

Behavioral assessments of ANU's vision (Table II) were interesting in that he was late to demonstrate fixation and some measures of responsiveness. In particular, he did not reliably show optokinetic nystagmus (OKN) until after 12 weeks. We normally find OKN by 3 weeks in this species. The development of nonvisual reflex and motor functions appeared to be normal.

Acuity, assessed at 7.5 weeks by FPL, was found to be similar for his two eyes, approximately 6/60 Snellen (Table II). This is on the poor side for monkeys of his age but not outside the normal range. The second acuity assessment at 11 weeks revealed a difference between the two eyes of about 0.6 octaves. Operant data are not yet available for this animal.

*Case 3.* Monkey JAX has a congenital exophoria. The deviation was first noted during the first week after birth, along with slight motor tremors. The phoria has been maintained as have the tremors. Refraction revealed no unusual error.

*Case 4.* Monkey SAY has intermittent esotropia that is of unknown onset. She was identified at the Field Station at 6 months of age. She has an unusually large hyperopia but normal-looking fundi.

*Case 5.* Monkey MAA has a right esotropia of unknown onset. She was identified at the Field Station at 5 years of age. She also has an unusually large degree of hyperopia but normal-looking fundi.

*Case 6.* Monkey RAF has an esophoria that was first noted 10 weeks postnatally. She was identified at the Infant Primate Facility. The phoria has been maintained, but no further assessments have been made.

*Case 7.* Monkey RAB had an incomitant esotropia and was identified at birth at the Infant

*A 1-octave change would be a doubling of Snellen acuity, e.g., from 6/6 to 6/12.

Primate Facility. The squint was accompanied by congenital cardiovascular anomalies, and he died 6 weeks postnatally.

• • •

The approximate incidence of strabismus in our colony was 2% of those screened. We are currently developing a series of screening tests based on clinical tests that will allow more careful examination of the colony. We expect that more affected animals will be identified with closer scrutiny.

We have examined the genealogies of the naturally strabismic monkeys and as yet have found no common parents. It is impossible to know whether there is a common ancestor beyond the parents because 5/7 of the mothers and 3/7 of the sires were wild-born. Of those with ancestors in the colony, there are none in common.

***Discussion.*** All seven monkeys described in the present report naturally developed a strabismus. Five were examined ophthalmoscopically, and no obvious organic cause was found in any case. Their cases are in many ways similar to human clinical cases. KAY's acquired incomitant esotropia occurred in conjunction with anisometropia and may be accommodative. The squint appeared at a time in her development that would be comparable to common onset in humans.* ANU's infantile comitant esotropia began as abnormal eye movements during attempts to fixate and became consolidated at a time when we find localization to ordinarily develop in this species. This form of strabismus is the most common form seen in human infants.[2, 4] SAY and MAA both have esotropia in the presence of a large refractive error. We do not know the histories of either animal, to be sure that the strabismus and refractive error are causally related. However, it is not uncommon for children with large refractive errors to develop strabismus.[2] It is important to note that none of these animals shows any other obvious sensory or motor abnormality. JAX, on the other hand, has some minor motor problems as well as the strabismus. His refractive error is not particularly unusual. These data suggest that his squint may be of motor rather than sensory origin.

One question to be addressed is why naturally strabismic monkeys have not been observed before. For our own colony, we feel that there are two reasons. The first is that strabismus is often not readily apparent by casual observation. Most monkeys older than about 4 months avoid eye contact with humans, making it difficult to determine eye alignment. In addition, many forms of strabismus are only apparent under specific viewing conditions. For example, target distance, accommodative state, and direction of gaze all may influence the appearance of a strabismus. The conditions at the Infant Primate Facility are rather unique in that the monkeys are housed in individual cages and are handled routinely. Thus it is relatively easy to observe the infants under a variety of conditions. The Field Station facilities, on the other hand, are less well suited for observation because of group housing and infrequent handling of the monkeys. But it is possible to observe them fairly carefully by the procedure described in Methods.

The second reason is the availability of improved nursery care. It is possible to successfully raise premature and low-birth-weight infants that may not have survived had they been left with their mothers.[7] Clinical data suggest an increased risk of strabismus with prematurity.[1] ANU, for example, was born 2 weeks prematurely. Cross-eyed monkeys may be less likely to survive in captivity, or in the wild, if left to fend for themselves.

The existence of naturally strabismic monkeys, which we have described, defends the utility of the macaque monkey as an animal model for human strabismus and amblyopia. This information argues against the contention that the macaque oculomotor system has the capacity to keep the eyes straight under all circumstances. The existence of these animals provides a natural nonhuman primate model for studying strabismus and amblyopia and factors that influence their development. In addition, these animals provide support for the macaque monkey in general as a good model for human visual and oculomotor disorders.

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*We find that 1 week of *Macaca nemestrina* visual development is approximately equivalent to 1 month of human visual development.[10] Thus comparable onset would be about 15 months.

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**Key words:** strabismus, monkeys, animal model

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## Monocular spatial distortion in strabismic amblyopia.

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*We examined monocular spatial vision of strabismic amblyopes by measuring errors of relative directional-ization (specifying whether or not two targets are in vertical alignment) and partitioning (equating left- and right-field spaces). Abnormally large errors were made when fixation occurred with the amblyopic eye; these errors are not attributable to reduced acuity, unsteady fixation, or eccentric fixation. From the results we infer that monocular space perception of strabismic amblyopic eyes is severely distorted and is characterized by "bending" of vertical lines of direction and by local "compressions" and "expansions" of horizontal spatial values. Such distortions can readily account for many of the oculomotor abnormalities of the amblyopic eye as well as for the strabismic subject's phenomenological description of the difficulties experienced in using this eye—difficulties that are typically much worse than the reduced acuity would predict.*

In their classic paper, Wald and Burian[1] proposed that the visual deficit of strabismic amblyopic eyes affects form or pattern vision with sparing of light perception and spatial projection. In order to assess form vision, Wald and Burian measured visual acuity for letters presented on a standard clinical chart. Pirenne's[2] subsequent analysis of visual acuity (resolution) as a special type of light difference discrimination raises doubt as to whether such visual acuity measurements adequately evaluate form vision.

A number of observations indicate that the visual deficit in strabismic amblyopia is not adequately specified in terms of acuity (or of grating contrast sensitivity, which has recently been proposed to supplant traditional visual acuity measures[3] but which is also readily subsumed by Pirenne's light-difference discrimination analysis). Measured visual acuity of amblyopic eyes typically shows considerable variability and depends, in part, on whether a full chart, single lines of letters, or isolated symbols are used.[4] Hess et al.[5] have presented contrast sensitivity curves for two strabismic amblyopic eyes that are indistinguishable from those for their subjects' preferred eyes. Indeed, grating resolution thresholds reported for strabismic amblyopic eyes are typically much better than expected from the performance of these eyes on letter acuity tests.[3] Clearly, the visual angle subtended by an acuity or grating target may not be the sole variable, or even the most relevant, in determining the visual performance of an amblyopic eye.

About 20 years ago, Pugh[6, 7] reported that amblyopes perceived distortions when they viewed test letters with the affected eye; their descriptions included abnormal spacing between letters or their parts, fragmenting of letter, and changes in the shapes of letters. Recently, Hess et al.[5] reported

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