ing agent. C-6 permitted the action of pilocarpine on the E_2 sites to become apparent as evidenced by a marked increase in aqueous humor formation. As stated in a previous publication,[4] the only known action of C-6 pertinent to these experiments, is its ability to block E_1 sites of ganglion-like receptors.

The ability of pilocarpine to stimulate E_1 sites was further indicated by its action in reversing the increased aqueous inflow, IOP, and C induced by Ach+Es. As reported earlier,[2] Ach+Es produce their effect by an action on E_2 sites while leaving the E_1 sites essentially unoccupied. It, therefore, seems quite probable that pilocarpine stimulated the free E_1 site receptors while the E_2 sites were bound by the acetylcholine.

Stimulation of E_2 sites of the uveal ganglion-like receptors by Ach+Es produces an increase in aqueous humor formation probably by vasoconstriction of efferent blood vessels of the ciliary processes to increase ultrafiltration pressure and ultrafiltration.[9] Pilocarpine, when administered together with C-6, is surmised to act in a similar way. It has been suggested[5] that some adrenergic amines which are capable of decreasing the Ach+Es elevations of inflow act on E_1 sites of ganglion-like receptors to produce vasoconstriction of afferent ciliary process blood vessels to decrease ultrafiltration. It is presumed that E_1 stimulatory properties of pilocarpine act in a manner similar to the adrenergic amines.

From the Clinical Branch, National Eye Institute, National Institutes of Health, United States Department of Health, Education and Welfare, Bethesda, Md. 20014. Submitted for publication April 8, 1974.

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Velocity characteristics of normal human saccades.

D. BOGHEN, B. T. TROOST, R. B. DAROFF, L. F. DELL'OSSO, AND J. E. BIRKETT.

There is lack of agreement concerning many velocity-amplitude characteristics of saccadic eye movements. We analyzed, in 15 normal subjects, factors such as abduction, adduction, centering, eccentric, and across-the-center refixations to determine their possible influence upon peak velocity (PV) for 5°, 10°, 20°, and 30° movements. There was considerable intra- and intersubject variability in PV at each amplitude. Analysis of variance revealed no statistically significant difference among the various types of saccades except for the average PV of adduction refixations which was significantly greater at the 30° amplitude. Comparison of conventional 25 Hz. bandwidth direct current electro-oculography (EOG) and 100 Hz. bandwidth infrared reflection showed higher values with the latter technique. We obtained normative data on saccadic velocity which permits definition of "pathologic slowness." The lower limits of normal mean PV for saccades of 5°, 10°, 20°, and 30° are 114, 167, 188, and 200° per second for conventional EOG and 145, 196, 213, and 227° per second for infrared reflection. Any mean PV which falls below these values should be considered abnormal.

Saccadic eye movements may be defined entirely on the basis of velocity-amplitude characteristics. Despite the obvious importance of peak velocity measurements, there is lack of agreement about saccadic velocity-amplitude relationships in general[1-5] and particularly with regard to direction of movement. Saccades variably have been described as being faster in the temporal (abduction) as compared with the nasal (adduction) direction[6, 7]; faster in the nasal direction[5]; equal in both directions[2]; and faster toward (centering)

Table I. Variation of Mean PV's in three Ss recorded in two sessions at an interval of several months

	Amplitude of movement							
	5°		*10°*		*20°*		*30°*	
Ss	**1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*
1	207	176	275	260	361	328	419	379
2	185	176	282	271	388	403	443	461
3	172	173	257	276	363	371	421	435

In the underlined pairs, significant differences existed between first and second recording sessions.
*First and second recording sessions.

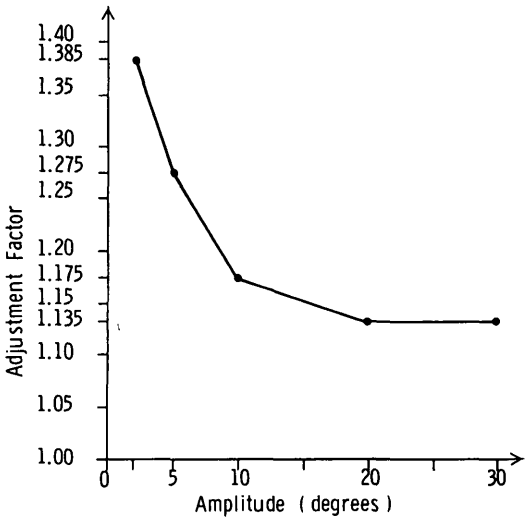

Fig. 1. Adjustment curve indicating the relationship between infrared reflection and conventional EOG techniques. More accurate saccadic peak velocities may be obtained from those measured using 25 Hz. bandwidth EOG by multiplying the latter by the adjustment factor indicated for each saccadic amplitude.

as opposed to away (eccentric) from the primary position.[2, 4]

Our study was prompted by the above uncertainties and also the need to investigate a sufficiently large sample of normal subjects to establish normative data which are required to adequately define pathologic slowness of saccades in patients with neurologic disease.

Methods. Fifteen normal persons (three females; twelve males), all right-eye dominant as determined by sighting tests, with 20/20 acuity, served as subjects (Ss). Thirteen were between the ages of 22 and 36, and the others were 56 and 75 years old. None of the Ss had used sedatives, hypnotics, or anticonvulsants within a period of at least one week preceding the study. The Ss sat in a modified dental chair with a bite plate for head stabilization and eye movements were recorded by direct current (dc) electro-oculography (EOG) in a manner previously described for this laboratory.[8] The left eye was occluded and only the right eye was recorded. The eye position signal was electronically differentiated to obtain peak velocity (PV). The full system bandwidth of this recording system was 25 Hz. with a response-time for the differentiator of 15 msec. Simultaneous recordings were also made using EOG and infrared reflection with the electronics modified to a full system bandwidth of 100 Hz. and a differentiator response-time of 4 msec. This enabled us to compare data recorded from a high bandwidth infrared system with that obtained by conventional EOG (Fig. 1).

Fixation targets were tungsten filament white bulbs 1 cm. in diameter which were masked with white paint to eliminate glare. They were mounted on a flat background at distances to subtend visual angles of 0°, 5°, 10°, 20°, and 30° to the right and left of the Ss right eye when the 0° light was 1.14 meters from the cornea.

Following calibration, the Ss executed refixations to the targets and back to center. In addition, refixations were made across-the-center in both directions. Each subject thereby performed temporal, nasal, eccentric, centering, and across-the-center refixations. Periodic verbal encouragement and frequent rest periods were given to insure alertness. Calibration was checked after each series of refixations and the approximate recording time for each subject was two hours. In each run, the subject executed as many refixations as necessary to obtain at least 20 accurate saccades, free of overshoots, undershoots, and blink artifacts. We could interpret the eye movement analogs with an accuracy of 0.5° for eye position and to 10° per second for velocity.

Results. By simultaneously measuring saccadic velocity with conventional low bandwidth dc EOG and infrared techniques, we determined an adjustment curve to arrive at more accurate values (Fig. 1). By inspection of the vertical axis, this curve provides the percentage by which low bandwidth data must be raised (e.g., the velocity of a 10° saccade must be raised by 17.5 per cent).

The velocity-amplitude relationship of the mean

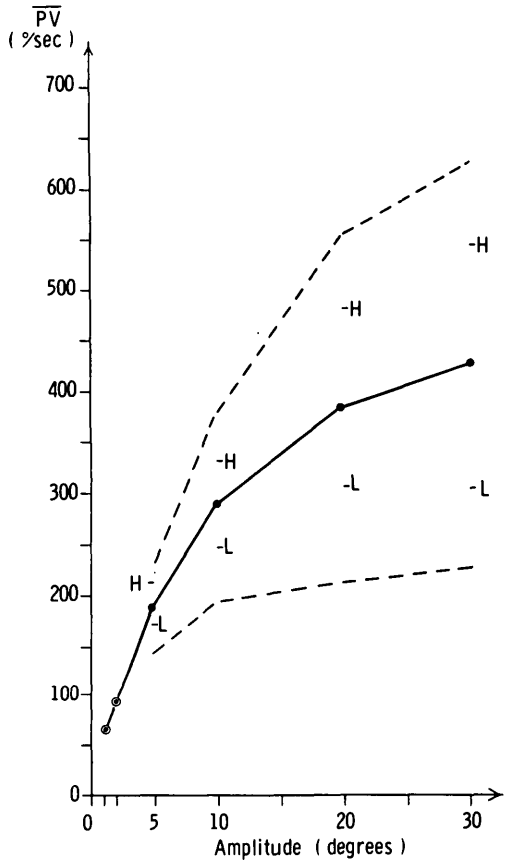

Fig. 2. Velocity-amplitude plot of mean PV values for 15 normal subjects. Dotted lines indicate the 95 per cent range of normal velocites. Highest (H) and lowest (L) individual means are indicated. The values shown for one and two degrees were obtained from two subjects. Actual mean PV values for 5, 10, 20, and 30° are 189, 288, 385 and 428 degrees per second, respectively.

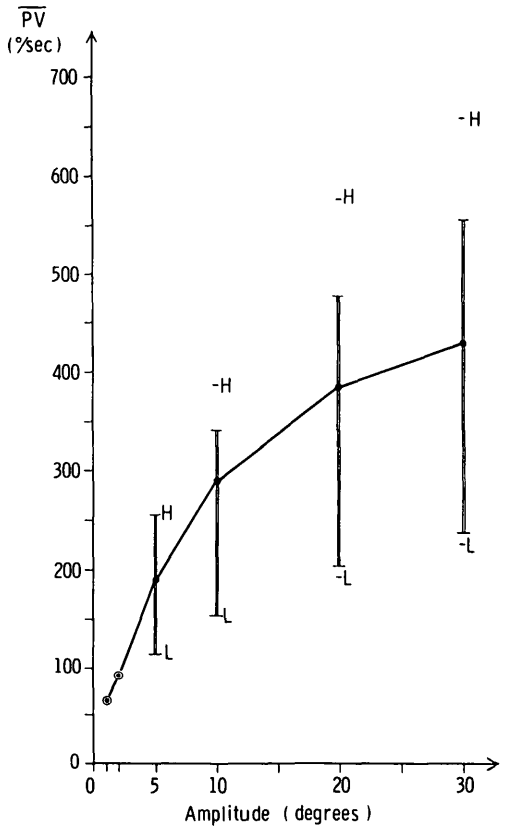

Fig. 3. Velocity-amplitude relation of mean PV values (as in Fig. 2) showing maximum single-subject range at each studied amplitude and the highest (H) and lowest (L) individual values recorded.

PV values in 15 normal subjects is shown in Fig. 2. The rate of increase gradually diminished with larger amplitude saccades, but analysis of individual records disclosed several exceptions. In three subjects, PV values remained unchanged between 20° and 30°; in two others, it increased at the same rate between 10° to 20° and 20° to 30°.

The PV values had considerable *intra*-subject variability, and the greatest single-subject ranges are plotted in Fig. 3. Three Ss had two complete recordings at intervals of several months (Table I); one had values with statistically significant differences at all amplitudes, whereas the differences in the other two depended upon the amplitude.

The analysis of variance showed no significant difference among velocities of centering, eccentric, and across-the-center refixations when the data from all Ss were combined. The PV of nasal saccades were significantly greater than those of temporal saccades ($P < 0.05$), but only at the 30° amplitude (mean PV of 30° nasal saccades equals 461° per second, mean PV for 30° temporal saccades equals 426 degrees per second). Six out of fifteen subjects had faster nasal saccades at 30° and two of these had similar differences at 20°. One additional subject had faster nasal saccades at 20° but not at 30°. No subject demonstrated faster temporal saccades.

Discussion. Previous studies of saccadic velocity in humans have utilized a variety of recording techniques and methodologies which might explain the differences in results. Techniques have included after-images, corneal reflection, reflection from a contact lens to photosensitive paper or to a fiber-optic system, high-speed photography,[1] alternating current and direct current electro-oculography,[1, 5, 7] contact lens scleral search coil in a magnetic field, and infrared reflection.[4, 7] The target displays have been fixed position,[1, 2, 6] computer generated "quasi-random" movements,[4]

or moving targets.[7] Several studies did not restrict velocity analysis to accurate saccades of specific amplitudes but averaged all values for a given intended refixation despite considerable differences in actual eye movement size.[7] Many studies included less than four subjects[1, 4, 6] and often only a few eye movements were recorded.

The intra- and intersubject variability found in our study establishes the necessity of recording a sufficient number of subjects and eye movements before accurate conclusions can be determined. Hourly fluctuations, such as attributed to alcohol,[9] may simply reflect intrasubject variability. We have determined that at least 10 accurate saccades of 5° or 10°, or 15 accurate saccades of 20° or 30° must be analyzed to be 95 per cent confident that the mean is within one standard deviation of the true mean.

As stated in the introduction, there has been disagreement concerning the relationship of movement direction to saccadic velocity. In approximately half of our subjects, nasal (adducting) movements were faster than temporal movements (abducting), but the difference was significant statistically only at the 30° amplitude. Only one of the three subjects who were studied on two occasions separated by several months demonstrated faster nasal saccades and this was present both times. We cannot readily explain our disagreement with those studies which found that temporal saccades were faster than nasal saccades.[6, 7]

The comparison of published results of velocity-amplitude relationships of saccades in previous studies[2, 4] indicates a considerable variation. Some of the differences might be explained on the basis of sample size but the most important factor is recording technique. Infrared methods generally give higher values than those measured by EOG. Furthermore, the electronics of the recording system is of extreme importance[6] and those studies utilizing higher bandwidths have yielded faster and more accurate velocities.

The increase in velocity of saccades with larger interfixational amplitudes has been long recognized and the nonlinearity of this relationship for large amplitudes had also been established.[2, 3] In the present study, the increase in PV for each degree of increasing amplitude is approximately 20° per second between 5° and 10° of amplitude, 10° per second between 10° and 20°, and approximately 4° per second between 20° and 30° of amplitude.

A major purpose of this study was to obtain normative data for saccadic velocities in order to define adequately pathologic slowness in patients with neurologic disease affecting the ocular motor system. We commented on the need for such information in a previous paper[10] where we were unable to state with certainty whether a patient with a cerebral hemispherectomy had normal or slow saccadic velocities. Any mean PV which falls below the 95 per cent confidence interval determined by our present study should be considered abnormal. Specifically, the lower limits of normal $\overline{PV}$ for 5°, 10°, 20°, and 30° saccades are: 145, 196, 213, and 227° per second. Normal subjects may have some individual PV values below these lower limits. These values are for our laboratory using an infrared recording technique with a 100 Hz. bandwidth. The $\overline{PV}$ using conventional dc EOG with a 25 Hz. bandwidth are 114, 167, 188, and 200, for 5°, 10°, 20°, and 30° saccades, respectively. Laboratories conducting clinical oculographic recordings are advised to establish their own normative values with their own recording technique and electronics.

From the Ocular Motor Neurophysiology Laboratory, Miami Veterans Administration Hospital and the Department of Neurology, University of Miami School of Medicine, Miami, Fla. Dr. Boghen's work was aided by the Canadian National Institute for the Blind by means of an E. A. Baker Foundation Grant for the Prevention of Blindness and an H. K. Detweiler travelling fellowship of the Royal College of Canada. Submitted for publication March 7, 1974. Reprint requests: Dr. R. B. Daroff, Veterans Administration Hospital, 1201 NW 16 St., Miami, Fla. 33125. A preliminary report of this work was given at the Association for Research in Vision and Ophthalmology meeting in Sarasota, Fla., May, 1973.

Key words: saccadic eye movements, saccades, velocity-amplitude characteristics, eye movement, EOG, infrared, eye movement velocity, eye movement recording.

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Epithelial cell phagocytosis of *Listeria monocytogenes* in the conjunctiva.

MATTHEW C. ZIMIANSKI, CHANDLER R. DAWSON, AND BIRGITTA TOGNI.

Guinea pig conjunctivas inoculated with Listeria monocytogenes *were studied by light microscopy and transmission electron microscopy. The epithelial cell of the conjunctiva was found to phagocytose* L. monocytogenes *as early as 15 minutes after inoculation, long before the appearance of polymorphonuclear cells. The epithelial cells of the mucous membranes appear to participate actively in the host response to this pathogenic organism.*

In extensive studies of phagocytosis, Metchnikoff[1] described two types of functionally important phagocytic cells. These he called "microphages" and "macrophages," neutrophilic or eosinophilic leukocytes acting as microphages, and large mononuclear cells acting as macrophages, a concept which has been widely accepted.[2]

One aspect of this concept that has never been fully explained is the role of the epithelial cell in the phagocytosis of parasitic microorganisms on mucous membranes. In 1921, Lindner[3] observed epithelial phagocytosis in the conjunctiva and his observations were extended by Howard.[4] A number of bacteria have been shown to be taken into epithelial cells, both in experimental and naturally occurring infections.[5, 6] The importance of epithelial cell phagocytosis in the host defense against infection has not yet been determined.

In rabbits and guinea pigs *Listeria monocytogens* produces a severe keratoconjunctivitis.[7] Typically, *L. monocytogenes* induces a monocytosis in the involved host and bacteria can be identified in some of these monocytes.[8] Recent studies

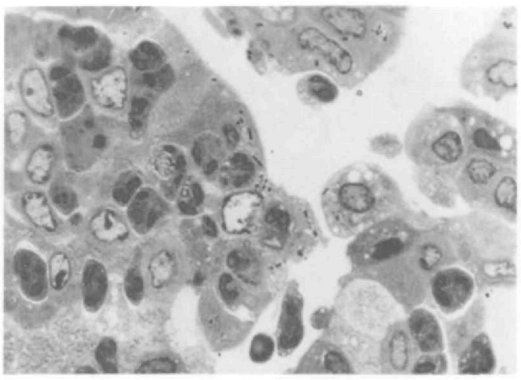

Fig. 1. Light photomicrograph of guinea pig conjunctiva 30 minutes after inoculation with *L. monocytogenes*. The dark granules in the cytoplasma of epithelial cells are bacteria, presumably from the inoculum. (×640, toluidine blue staining.)

by Racz and co-workers[9, 10] have shown that *L. monocytogenes* penetrates into epithelial cells of the cornea, intestine, and bladder. In order to study the role of conjunctival epithelial cell phagocytosis in *Listeria* infection, we have made sequential observations by light and electron microscopy following inoculation of the guinea pig conjunctiva with this organism.

Colonies from 48-hour cultures of an *L. monocytogenes* strain* were inoculated onto the superior and inferior palpebral conjunctiva of albino guinea pigs with a sterile cotton-tipped applicator. After intervals of 15, 30, 60, 90, and 120 minutes, and 4 hours the animals were killed and specimens were obtained.

For light and transmission electron microscopy the superior and inferior palpebral conjunctivas were removed in toto and immersed immediately in cold, buffered, 3 per cent glutaraldehyde at a pH of 7.4. A 2 mm. central strip of conjunctiva was postfixed for two hours with 2 per cent osmic tetroxide in veronal acetate buffer, and embedded in araldite epoxy resin. Sections 1 to 2 μ thick were cut for light microscopy and stained with basic fuchsin and methylene blue. Thin sections stained with uranyl acetate and lead citrate were examined with an RCA electron microscope.

Adherence of bacteria to epithelial cells. In electron photomicrographs, bacteria were found at the exposed surface of conjunctival epithelial cells at 15, 30, and 90 minutes after inoculation even though the tissue had been thoroughly washed and rinsed during processing (Figs. 1 and 2). In control (noninoculated) conjunctivas no bacteria were found at the cell surface or within

*Obtained from the Department of Microbiology, University of California, San Francisco.