data demonstrate the feasibility of mapping polymorphic restriction fragments from HLA recombinant families.

With the exception of n, all of the polymorphic restriction fragments segregated with parental HLA haplotypes; this result is consistent with linkage of the segregating genomic fragments to the HLA region. Although classical linkage analysis (lod scores) for a particular fragment cannot be applied to these data, the likelihood (*11*) that by chance alone all except band n of the polymorphic fragments identified here would have shown intrafamilial distributions consistent with HLA haplotype segregation is $(3/64)^3 \times (5/64)^3 \times (3/16)^{11} \times (5/16)^4 = 4.7 \times 10^{-18}$. This result is consistent with the finding of Erlich *et al.* (*12*) that all of the class I genomic fragments are localized on a discrete region, defined by a deletion mutant, on the short arm of chromosome 6. In one family, however, Pvu II digestion revealed the presence, in one child, of a new fragment apparently resulting either from the loss of a Pvu II site by a serologically undetected mutation or from a recombination outside the region detected by serologic and enzymatic markers. The presence of a novel 3.4-kb fragment as well as the absence of a 3.3-kb and a 0.8-kb fragment has been confirmed in the analysis of DNA from an Epstein-Barr virus–transformed cell line derived from this individual. The absence of these two fragments could be attributed to segregation (with the A and C haplotypes), in which case their absence in DNA from the child (haplotype B/D) would be unrelated to the appearance of the novel 3.4-kb fragment. Alternatively, the absence of these fragments might have resulted from the event that gave rise to the new fragment. Recent data on the amino acid sequence of mutant class I antigens (*13*) and nucleotide sequence analysis of mutant genomic clones (*14*) have led to the hypothesis that an event analogous to gene conversion in fungi may be involved in the generation and maintenance of MHC polymorphism (*13, 14*). It is possible that the newly arisen Pvu II fragment observed in this pedigree may have resulted from such an event.

The study of restriction endonuclease fragment polymorphism in families has yielded several important findings. First, the generation of an apparently new fragment, an event revealed by comparing parental and progeny patterns, was observed in family Stk. Second, recombinant haplotypes detected serologically, as in family Riv, have been useful in mapping polymorphic restriction fragments. The analysis of such crossover families can also help to define the specificity of putative locus-specific probes. Third, the segregation analysis of genomic fragments described in this report shows that the genes homologous to the probe we used are part of the HLA region. This methodology is a powerful new tool for the study of MHC gene organization and polymorphism. The DNA polymorphisms defined in these analyses are likely to subdivide some serological specificities and to be correlated with others. Their continued study may help to elucidate the nature of susceptibilities to HLA-linked diseases.

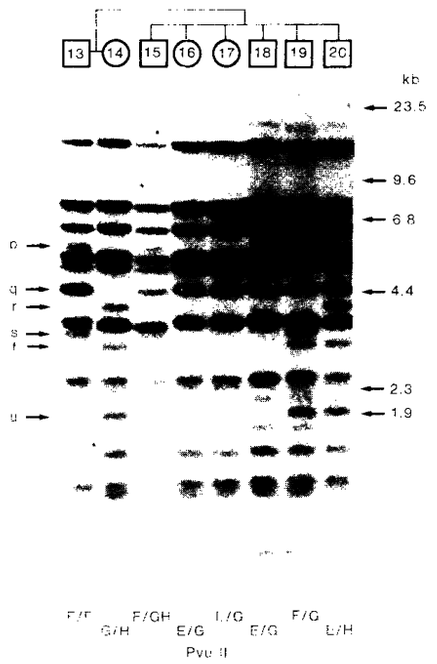

Fig. 2. Genomic blot analysis with class I probe of Pvu II–digested DNA from family with HLA crossover. DNA samples were prepared, digested with Pvu II, and analyzed as described in the legend to Fig. 1. The HLA haplotype of the father is designated *E/F* and that of the mother *G/H*. DNA from the child with the recombinant haplotype (*GH*) is in lane 15. The slightly increased mobility of fragment r in lane 15 relative to its mobility in lanes 14 and 20 is probably due to the slightly decreased amount of genomic DNA located in lane 15.

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19. We thank S. Weissman for making the HLA-B7 clone available. Supported in part by Cetus Corporation and by NIH grant HL29572.

2 May 1983, revised 18 July 1983

Eye Movements of Preschool Children

Kowler and Martins (*1*) have reported that the eye movements of two preschool children are considerably less accurate than adult eye movements under identical viewing conditions. Although we do not dispute the validity of the eye movement recordings obtained from these children, we do disagree with the interpretation that normal children are significantly deficient in oculomotor control and with the conclusion that these presumed deficiencies "limit a child's ability to use eye movements to acquire visual information" (*1*, p. 997).

1) Young children are notoriously poor at maintaining attention during a task, even for brief periods. Despite the authors' opinion that the children were attentive and cooperative, the children's expectations for accurate performance, as well as their level of attention and motivation, were likely to have been lower than the adults'. Furthermore, studies of visual abilities in preschoolers have shown that training and feedback are essential to optimize performance (*2*). Thus, although the two children showed larger mean saccade vector magnitudes during fixation of a stationary target than the adult did, this difference

in average performance was largely the result of greater variance in the saccade magnitudes. Similarly, although both the children and adult showed mean drift velocities centered around 0 minutes of arc per second, the variance of these drift velocities was greater in the children than in the adult. Both of these instances of greater variance can be most plausibly accounted for by limitations of attention rather than capacity.

2) Only two children were tested, and their performance was compared with that of a single adult subject. Given this small sample size, it is critical to document the absence of visual abnormalities in each subject so that their performance can be generalized to the larger population. The authors did not report several key visual characteristics of the children, such as the presence of strabismus, nystagmus [possibly present in the record of Philip in their figure 1B (*1*)], or refractive errors. These visual anomalies can significantly degrade fixational ability (*3*).

3) The authors say that "The children's high retinal image velocities may impair vision. Their large saccades introduce fixation errors which may also impair vision" (*1*, p. 998). These statements are misleading for several reasons. It appears from their figure 2 (*1*) that approximately 90 percent of the time both of the children showed saccadic magnitudes and drift velocities no greater than 1 degree and 1 deg/sec, respectively. Saccades of 1 degree do not significantly impair either visual acuity or more complex aspects of visual processing (*4*). Although saccades in children may be slightly larger while they are fixating a small spot on an oscilloscope screen, it is unlikely that oculomotor control per se significantly impairs the child's visual processing of more complex displays (*5*). In addition, the authors themselves noted that retinal image velocities less than 2 deg/sec do not impair visual performance in adults (reference 13 in (*1*). Thus, the implication drawn by the authors that the fixational movements made by preschool children are likely to impair visual processing is unfounded.

Kowler and Martins (*1*) failed to consider a number of key issues in collecting and interpreting eye movement data from two preschoolers. The conclusions that preschool children are in general deficient in basic oculomotor abilities, and that these presumed deficiencies significantly impair their ability to process information in visual displays is unjustified. Although young children may have subtle performance deficits on several oculomotor tasks, their optimal performance as shown by Kowler and Martins resembles that of untrained adults.

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1 April 1982; revised 28 December 1982

Kowler and Martins (*1*) reported immaturities of oculomotor control in two normal children 4 and 5 years old. They suggested that such deficits might also be present in young infants and, further, that these deficits might cause the observed discrepancy between measures of infant and adult spatial contrast sensitivity (*2*). We disagree with the latter hypothesis.

The children in the Kowler and Martins study exhibited two specific oculomotor immaturities relevant to our discussion. (i) When fixating a stationary point, the children showed larger off-target saccades than the adult did, and (ii) their retinal image velocities during saccade-free periods were more rapid than the adult's. Kowler and Martins hypothesized that young infants may exhibit similar or even more pronounced oculomotor deficits but did not present any supporting eye movement data from infants. They made two specific arguments of how such deficits could reduce young children's and infants' spatial contrast sensitivity.

1) Children's (and infants') larger off-target saccades may limit their acquisition of visual information because such saccades would place the target off the fovea. This argument is implausible, however, because most studies of infant pattern vision have used large stimulus displays (*2*).

2) Kowler and Martins suggested that "high retinal image speeds in infants could account for the shape of their contrast sensitivity function" (*1*, p. 998). The adult contrast sensitivity function (CSF) peaks below 1 cycle/deg with high drift velocities under stabilized retinal image conditions (*3*). The peak of the young infant's CSF is also at about 1/2 cycle/deg, under normal viewing conditions (*2*). Kowler and Martins suggested from this correspondence that the shape of the young infant's CSF might be due to the supposed high-speed shear between the target image and the infant's retina caused by high retinal-image velocities. We examined this hypothesis quantitatively; to shift the peak of adult CSF's to the 1/2 cycle/deg peak of the CSF of a 1- to 3-month-old infant, average retinal image velocities of 960 minutes of arc per second would be required (*4*). This value is unreasonably high given what is known of infant eye movements (*5*).

The claim that the shape of young infants' CSF's results from oculomotor immaturities alone is unwarranted. The change in shape with age (particularly, the peak shift) is more plausibly attributed to postnatal changes in retinal anatomy (*6*) and to progressive narrowing in the spatial tuning of pattern mechanisms (*7*).

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4. From figure 11 of Kelly (*3*). We conservatively used angular rather than linear image velocity across the retina in our calculations. Equating linear image velocities across the retina would require a much greater angular image velocity for infants because of the shorter axial length of infant eyes [J. S. Larsen, *Acta Ophthalmol.* **49**, 441 (1971)].
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12 April 1982; revised 20 December 1982

Neither Aslin and Ciuffreda nor Dannemiller *et al.* offer convincing reasons for altering our two main conclusions, namely that (i) eye movements of children differ from eye movements of adults and (ii) these differences have implications for understanding visual development (*1*).

Aslin and Ciuffreda suggest that our subjects were inattentive and uncooperative. This was not the case. Their attention and cooperation was demonstrated

by their excellent, adultlike performance in many tasks. For example, the accuracy and latency of the saccades they used to track low-frequency (0.4 step per second) periodic target steps (figure 1C in *1*), the gain of their smooth pursuit (figure 1E in *1*), and the frequency of their saccades made during fixation of the stationary target (*1*, p. 998) were the same as those of the adults. These results show that the children were attentive and followed the instruction to look at the target both when it was stationary and when it moved.

The observed differences in the smooth eye movements of children and adults [that is, the children's higher smooth eye speeds during fixation of the stationary target (figure 2B in *1*) and their longer lags when the target changed direction during smooth pursuit (figure 1E in *1*)] could not have been produced by failure to cooperate: among adults, neither smooth eye speed during fixation (*2, 3*) nor the direction of the eye during smooth pursuit (*4*) are under voluntary control. In addition, Aslin and Ciuffreda assume that precise control of saccade size and timing—observed in adults during target step-tracking (*2, 5*) and during fixation (*6*), but absent in the children (figures 1D and 2A in *1*)—requires training or special effort on the part of adults. On the contrary, performance of experienced and inexperienced adult subjects in these tasks is the same (*2, 5, 6*). Thus, the children's saccades could not resemble those of "untrained adults" because training of adults is not required to achieve highly precise control of saccades. Of course, a training procedure to teach children better control of saccades might be successful (*7*). Such an outcome would follow from our suggestion that children have difficulty with the precise control of saccade size and timing because they have not yet learned efficient oculomotor habit patterns (*8*).

Aslin and Ciuffreda are also concerned with possible idiosyncratic characteristics of our subjects. The subjects were and continue to be free of any visual or oculomotor abnormality, including the need for corrective spectacles. Also, our conclusions were based on a comparison of the children's performance with that of the many adult subjects who have been tested in studies that used eye monitors as accurate as ours (reference 2 in *1*). The performance of the inexperienced adult subject we reported was representative of the performance reported in prior studies (*2–6*).

Our conclusion that eye movements of children differ from those of adults is also supported by recent measurements (*9*) showing that the oculomotor performance of 10-year-old children falls between that of 5-year olds (*1*) and adults (*2–6*).

Aslin and Ciuffreda also suggest that the preschool children's saccades were too small to impair vision. The studies they cited showed that average saccade size does not predict visual acuity in clinical populations (*10*)—a result that was attributed to the patients' decisions to make their visual judgments while the target was optimally placed within the fovea (*10*, p. 1678). Thus, this study showed only that visual acuity is not impaired during intervals between large saccades. It does not contradict our conclusion that large saccades are harmful when they place the visual stimulus in a retinal location sufficiently eccentric [$\geq$ 12 minutes of arc (*11*)] to impair visual acuity (*11*), well within the range of sizes of the children's saccades. Thus, harmful effects of large saccades would not be expected for resolution of a large pattern of bars (reference 13 in *1*), but would be expected for tasks requiring precise control of saccades (*12*). The high retinal image speeds of the children, like their large saccades, may not affect performance on some visual tasks but may impair or even aid performance of others (*13*).

The computations of Dannemiller *et al.* incorporate a contribution of eye movements to visual development. Dannemiller *et al.* suggest that if retinal image speeds in infants are less than about 16 deg/sec, and if the sensitivity of the infants' visual system to retinal image motion is identical to that of adults, retinal image motion cannot account for all (but presumably can account for some) of the shift in the peak of the infant's contrast sensitivity function. It seems unlikely to us, however, that the sensitivity of neural mechanisms to spatial patterns changes with age while the sensitivity of the same mechanisms to image motion does not (*14*). Also, the studies Dannemiller *et al.* cited of eye movements of infants are inadequate to estimate even the upper limit of their retinal image speed because the electrooculogram (EOG) measures position of the eye relative to the head, not retinal image position. Since the velocity of the retinal image (R) is equal to the velocity of the eye relative to the head (E) plus the velocity of the head (H) when the target is more than 10 m away, an overestimate of E will lead to an underestimate of R whenever H is faster and opposite in direction to E. Overestimates of E are likely because of noise and baseline drifts in the EOG recordings and because of behavioral calibration procedures that assume perfect performance. For example, Tronick and Clanton's (*15*) calibration procedure may have overestimated E because they assumed that the gain of the infants' vestibulo-ocular response is *1*, whereas research with adult subjects has shown that gain is not 1 (*16*). When the target is less than 10 m away, R is no longer the sum of $H + E$ because the rotational axes of the head and eye are noncoincident (*17*). Nothing can be inferred about infants' retinal image speed from Aslin and Salapatek (*18*) because they neither calibrated their EOG recordings nor measured head movement.

Dannemiller *et al.* may believe that we ruled out a contribution of neural maturation of the visual system to the development of visual performance. This was not the case. We concluded that eye movements contribute to visual development—a possibility previously neglected by investigators of visual development (reference 14 in *1*) and now made plausible by our findings that eye movements are not fully developed by age 5 years.

Quantitative determinations of the effects of eye movements on visual processing in infants and children will require simultaneous measurement of both visual performance and retinal image position and velocity. This is necessary to find out how the eye movements of children affect their visual processing and whether effects of retinal image position and velocity on vision change with age. Such experiments may be technically difficult in infants but not, as we demonstrated, in children, who are equally valuable to study in light of recent reports that visual acuity is not fully developed until age 5 or later (*19*).

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15 August 1983

Co-Release of ACTH and β-Endorphin Immunoreactivity in Human Subjects in Response to Central Cholinergic Stimulation

We have reported that physostigmine, an acetylcholinesterase inhibitor with both nicotinic and muscarinic cholinomimetic properties, increases plasma levels of cortisol and β-endorphin immunoreactivity in human subjects (*1*). In our manuscript we commented that we were surprised that there was no significant correlation between increases in plasma concentrations of cortisol and β-endorphin immunoreactivity, since co-release of adrenocorticotropic hormone (ACTH) and β-endorphin from the anterior pituitary was observed in animal stress paradigms (*2*). However, we have recently measured plasma ACTH immunoreactivity concentrations in our original plasma samples using a newly developed assay (*3*), and we have found significant and highly correlated elevations in β-endorphin and ACTH immunoreactivity in plasma (Fig. 1). These results with physostigmine are now consistent with recent reports of apparent concomitant release of ACTH and β-endorphin in man in response to other stimuli including hypoglycemia, Pitressin administration and other conditions (*4*). Changes in plasma concentrations of ACTH and cortisol immunoreactivity were only weakly and nonsignificantly correlated.

In fact, more than 10 years ago Krieger and associates reported that while there was a close temporal correlation between plasma ACTH and corticosteroid peaks, there was no apparent proportionality between spontaneously released plasma ACTH and corticosteroid levels either within or among individuals (*5*). This lack of proportionality may be reflective of differing adrenal receptor sensitivities to ACTH, or differences in metabolic clearance rates of plasma ACTH and cortisol. Krieger has also suggested that the magnitude of corticosteroid response to ACTH is in part dependent on the recent history of prior adrenal exposure to ACTH. In addition, Holaday and associates (*6*) have reported a synchronized ultradian cortisol rhythm in monkeys which persists during supramaximal infusions of ACTH and suggested that bursts of cortisol secretion are not entirely dependent upon an immediately preceding release of ACTH.

Our new data suggests that central cholinergic stimulation may in part modulate the co-release of ACTH and β-endorphin immunoreactivity, and that in this paradigm, and possibly in others, plasma cortisol values may not be a good reflection of concomitant ACTH changes.

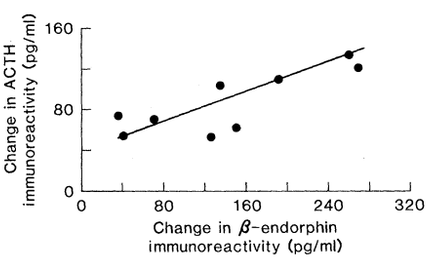

Fig. 1. Scatter diagram of maximal changes in plasma β-endorphin and ACTH immunoreactivity following physostigmine administration in nine subjects. Preinfusion values of plasma β-endorphin and ACTH immunoreactivity were compared with twenty minute postinfusion values. This postinfusion time point reflects peak increases in the individual hormones. Linear regression analysis was performed to determine the relationship and significance of physostigmine induced changes over time in plasma β-endorphin and ACTH immunoreactivity within individual subjects. ($r = 0.85$, $P < 0.01$)

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3. Samples were collected as previously described (*1*). All samples were assayed in duplicate within the same assay using an equilibrium radioimmunoassay utilizing a rabbit antiporcine ACTH antibody from Immunonuclear Corporation. The antibody has less than 0.1 percent cross-reactivity with α-MSH, β-endorphin, β-lipotrophin, leucine and methionine enkeplalin, bombesin, parathyroid hormone, prolactin, somatostatin, neurotensin, thyroid stimulating hormone, calcitonin, follicle stimulating hormone, leuteinizing hormone, human growth hormone, and substance P. Within-assay variability for ACTH is approximately 10 percent and sensitivity is 2 pg/ml. β-Endorphin/β-lipotrophin and cortisol values were determined as previously described (*1*). Linear regression analysis was performed to determine the relationships between physostigmine induced changes in plasma β-endorphin/β-lipotrophin, cortisol, and ACTH immunoreactivity across individual subjects.
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30 July 1981