proteins precipitated with IgG_1 κ revealed a protein that comigrates with a known structural protein of HSV-1 (55,000 daltons) (Fig. 1a).

With [^{14}C]glucosamine labeling, the monoclonal antibody was found to react with two glycoprotein bands, designated gD and pgD, the latter being the precursor of gD (Fig. 1b). Immunoprecipitation with HSV-2–infected cells showed that the specificity encompasses the two serological types of HSV (Fig. 1a), as already described by Eisenberg *et al.* for several mouse monoclonal antibodies against HSV (*11*). The antibody production has remained stable for a 2-year period, and the antibody specificity is preserved after freezing.

These results indicate that it is possible to obtain EBV-transformed bone marrow cells that continuously secrete a high and stable amount (15 μg per 10^6 cells per 24 hours) of human antibodies. Since few human myeloma lines are available for producing Ig-secreting human-human hybridomas, the transformation of human immune B lymphocytes by EBV appears to be a good approach to the production of human monoclonal antibodies. In general, lymphoblastoid cell lines from peripheral blood secrete polyclonal antibodies (*12*), but cloning should allow monoclonality. Since the correlation between the distribution profile of the cytoplasmic Ig cells and the levels of various Ig's in the serum is better in the bone marrow than in the other peripheral lymphoid tissues, the bone marrow, whenever it is available, seems to be a good source (*7, 13–15*) for establishing antibody-secreting lymphoblastoid cell lines. Such a line could not be obtained from peripheral blood lymphocytes. The small number of T lymphocytes in bone marrow may facilitate establishing cell lines. As has been observed recently (*16*), bone marrow contains more committed B cells than blood which contains more virgin B cells.

It is possible that oligoclonal cell selection in the bone marrow had occurred in vivo as a result of the immunodepressed status of the 73-year-old cancer patient. Alternatively, a premyelomatous state might have begun at the time we obtained the bone marrow. We cloned this Ri-BM cell line in vitro; most of the clones were secreting but not at a high level. The HSV antigenic stimulus probably occurred outside the bone marrow, since there is no local induction of antibody formation in the bone marrow but an immigration of antigen-activated B cells. From the 32 human lymphoblastoid cell lines that we have now established from HSV-positive donors, we have just obtained a second cell line producing a monoclonal antibody to HSV gD. Recently, the gene of this protein was mapped and a gD-related polypeptide was synthesized (*17*). A murine gD monoclonal antibody protects mice against HSV-induced neurological diseases (*18*) and thus a human gD monoclonal antibody that protects against both HSV-1 and HSV-2 might provide a means of passive immunization against HSV infections in humans.

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Interaction of Human Hemoglobin and Its Variants with Agar

Abstract. *In citrate agar electrophoresis hemoglobin appears to bind reversibly to the sulfated polysaccharide agaropectin, a natural component of Difco Bacto-Agar. This complex migrates anodally, since the hemoglobin is only weakly positively charged at pH 6.2 whereas the carbohydrate carries a net negative charge. Electroendosmosis, on the other hand, proceeds in the cathodal direction. These opposing fluxes separate the hemoglobins in the order of their affinity for agaropectin. An agaropectin binding site was identified on hemoglobin by computer-assisted modeling, and the relation of the site to hemoglobin variants that exhibit abnormal citrate agar mobility was established. The citrate anion is postulated to function as a "counter ion." Preliminary evidence indicates that agaropectin has antigelling properties with respect to hemoglobin S.*

Citrate agar electrophoresis is widely used for confirming the occurrence of hemoglobin (Hb) S (β6 Glu → Val) and Hb C (β6 Glu → Lys) in the presence of other Hb's and for differentiating these Hb's from variants with similar migration properties on other electrophoretic media (*1*). The method is based on the observation of Robinson *et al.* (*2*) that Hb migration on agar gels at *p*H 6.2 in the presence of citrate anions appears to depend on some factor other than, or in addition to, electrostatic charge. The order of Hb separation, cathode to anode, is F, A (and most variants of A), S, and C (Fig. 1a). Gratzer and Beaven (*3*), who studied the effect of a number of variables on the separation, concluded that electroendosmosis, the electric current–induced movement of buffer in a gel containing an immobilized charge, was probably the driving force and noted that the order of separation resembled the affinity of the Hb's for anionic ion-exchange columns (*4*). In addition, they suggested that the amino terminal of the β chain was involved. Schneider and coworkers (*5, 6*) made some generalizations about the relation between the structure of the Hb variants and their

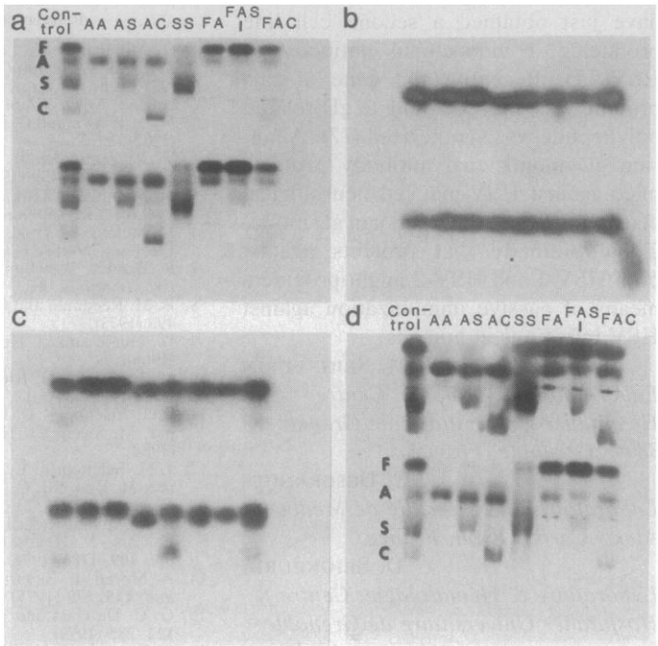

Fig. 1. Citrate agar gel electrophoresis of various oxyhemoglobin mixtures. The current was maintained at 50 mA for 45 minutes. All gels were prepared with 0.05*M* citrate buffer (*p*H 6.2) and stained with *o*-dianisidine. (a) Normal 1 percent Difco Bacto-Agar, (b) 0.5 percent agarose gel, (c) 0.5 percent agarose gel with 260 μg of partially purified agaropectin per milliliter, and (d) 0.5 percent agarose gel with 300 μg of λ-carrageenan per milliliter. The pale staining and diffuse nature of the bands in (b) are probably due to the absence of binding to sulfated polysaccharide. In pure agarose the hemoglobin bands stain pale brown with *o*-dianisidine, whereas in the presence of the sulfated polysaccharide the staining is blue. Diffusion and staining in (c) are intermediate between those in (a) and (b). The cathodal edge in (d) is brown-staining and diffuse because of electrophoretic depletion.

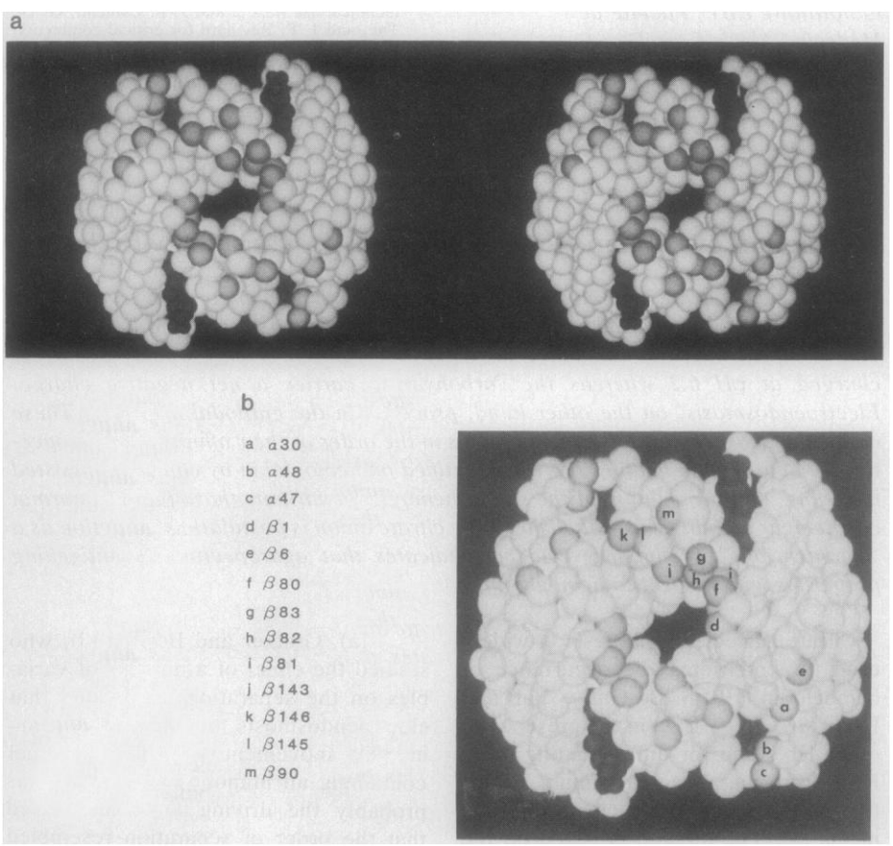

Fig. 2. (a) Stereo photograph of the computer-generated model of human deoxyhemoglobin. The molecule is viewed from the "bottom," or β-β, end, looking up the dyad axis. Each sphere represent one amino acid. The darker spheres are amino acids which, when altered by genetic mutation, yield hemoglobin whose migration in citrate agar electrophoresis is abnormal (dissimilar from that of Hb A). Additional dark spheres are present but are hidden from view in this projection. All occur in the region of the β-β cleft, however. (b) Key to the positions visible in (a).

mobility on citrate agar. While all these authors seem to agree that the β amino terminal plays a crucial role in the separation and that buffer movement in the gel is involved, we know of no complete explanation for the phenomenon. The earlier reports and our own experience led us to suspect an interaction between Hb and agar.

In preliminary experiments we sought to confirm the reports that only crude agar is effective in bringing about separation. Electrophoresis of Hb samples was carried out in agarose gels made with 0.05*M* citrate buffer at *p*H 6.2. As expected, neither separation of the Hb components nor migration of the Hb was observed (Fig. 1b). The fact that agarose was completely ineffective in promoting the separation suggests that some impurity or component of agar other than the principal gelling component is involved in the separation. Crude agar contains two primary components, agarose and agaropectin (*7*). Agarose, a copolymer of D-galactose and 3,6-anhydrogalactose, is the main gelling component and is uncharged at *p*H 6.2 (*8*). Agaropectin is believed to have a carbohydrate backbone similar to that of agarose, with roughly every tenth residue containing sulfate in *O*-sulfate linkages (*7*), and is the source of the negative charge in agar. Agaropectin is probably smaller than agarose and is loosely bound in the agarose gel framework. By itself it does not gel, presumably because of electrostatic repulsion.

To test the role of electroendosmosis in the cathodal migration of Hb F, polyethylene glycol (PEG)-6000, a neutral polymer of ethylene glycol, was applied at the origin of a conventional gel. After 45 minutes of electrophoresis, the PEG band was visualized by dipping the gel in 10 percent trichloroacetic acid. This neutral band migrated to essentially the same position as Hb F, indicating that migration of Hb F is due almost solely to electroendosmosis. Little or no electroendosmosis was observed in commercial agarose. Next, 1 percent agar solutions were melted and dialyzed while hot against citrate buffer. The dialyzed agar showed no loss of ability to separate Hb's, suggesting that a high molecular weight component such as agaropectin was involved. We then exhaustively electrophoresed agar gels with no Hb sample applied in order to remove charged, nongelled species. The electrophoresed gels were melted and cast into new gels. When tested, they were found to act in essentially the same manner as agarose, promoting neither migration nor separation. When the anode buffer,

which must contain all negatively charged molecules, was dialyzed, concentrated, and added back to commercial agarose, the properties of the original gel were partially restored (Fig. 1c). In a separate experiment, agaropectin was partially purified by precipitating the agarose with PEG-6000 (*9*). This component, when added to commercial agarose, also exhibited restoration or partial restoration of the capacity for Hb separation. Similar results were obtained when commercially purified λ-carrageenan, an agaropectin-like carbohydrate extracted from Irish moss, was included in agarose gels (Fig. 1d).

Thus it seems that agaropectin is responsible for both the anodal and cathodal migration, although by different mechanisms. The key to this dual function of agaropectin is that it can migrate in the agarose matrix with a mobility less than that of buffer ions. It therefore behaves as an immobile charge with respect to the buffer and causes electroendosmosis. We postulate that, simultaneously, Hb binds reversibly to agaropectin, forming a complex that moves slowly toward the anode. Free Hb would be carried toward the cathode by electroendosmosis. The separation thus reflects differences in the affinity of Hb's for agaropectin.

If Hb's A, S, and C bind differently to agaropectin, then a definite binding site should exist on Hb and should be close to the β6 position. To explore this possibility, we prepared a comprehensive list of Hb variants known to differ in their mobility in agar (*10*). A computer program that displays color views of Hb space-filling models in stereo pairs was used. The positions that affect agar mobility were displayed in a color different from that of the rest of the chain, and 18 views of Hb tetramers and dimers were projected on the monitor. These were photographed and examined with stereo viewing equipment. It was immediately obvious that all sites that affect agar mobility lie in a region that includes the β cleft as well as some parts of the α and β chains that are immediately adjacent to the cleft (Fig. 2). Moreover, the variants that rendered this site more negative (less positive) moved more like Hb F, that is, in the electroendosmotic direction. Those that rendered the site more positive (less negative) moved more like Hb C, or in the anodal direction. This explains the paradoxical observation that most variants that behave like Hb F (that is, are displaced toward the cathode) are also more negatively charged. The proposed binding site includes, but is larger than, the 2,3-diphosphoglycerate binding site. Therefore, if agaropectin binds preferentially to Hb S as suggested, it would be expected to lower the oxygen affinity [increase the partial pressure of O_2 at which hemoglobin is half-saturated (P_{50})] of Hb S but not of Hb F, since the latter does not appear to bind agaropectin. Crude agaropectin (2 mg/ml) nearly doubled the P_{50} of Hb S but increased the P_{50} of Hb F only slightly, as expected. The impact of the mutation at β6 on agaropectin binding may also be influenced by a conformational change near the amino terminal which results from the mutation. Such a conformational change has been shown to occur and to alter the affinity of Hb S for 2,3-diphosphoglycerate (*11, 12*). A puzzling feature of citrate agar electrophoresis is the requirement for a *p*H of 6.2. This is probably explained by the fact that histidines β2, 143, and 146 are a major source of positive charge in the cleft and, in order to be charged, would require such a *p*H.

Because citrate anions also bind at the 2,3-diphosphoglycerate binding site (*13*), citrate, aside from its role in maintaining the desired *p*H, may modulate the binding of Hb to agaropectin. This was borne out when acetate buffers at the same *p*H were used in place of citrate. Hemoglobin F moved with the electroendosmotic flow as usual, but Hb's A, S, and C migrated much closer together in a fashion roughly like that of Hb C. We therefore propose the following mechanism to account for the ability of citrate agar electrophoresis to separate Hb's. An agar gel is composed of a rigid agarose matrix in which agaropectin is loosely trapped, promoting cathodal electroendosmosis when electric current is applied. Agaropectin will move slowly toward the anode by electrophoresis. If an Hb mixture is applied to the gel, components (such as Hb F) that have little or no ability to bind to the agaropectin will be carried toward the cathode by the buffer flow. Other Hb's may be viewed as existing in an equilibrium between agaropectin-bound and -unbound factors. Citrate anions modulate this equilibrium by competing with agaropectin for the binding site. Thus the position that these Hb's assume in citrate agar electrophoresis reflects their affinity (at a constant citrate concentration) for agaropectin. This mechanism is sufficient to explain all major properties of citrate agar electrophoresis, including the effect of Hb concentration on the separation, since that, too, will affect the equilibrium.

Since agar gel electrophoresis is normally carried out at the atmospheric partial pressure of O_2 (PO_2), the validity of using a model of deoxyhemoglobin to study the agaropectin binding site may be questioned, especially in view of the location of the proposed binding site. The effect of agaropectin on the oxygen affinity reported above suggests that agaropectin does indeed bind preferentially to the *T* (tense or deoxy) state. Since the *T* state is in equilibrium with the *R* (relaxed or oxy) state, such a preferential binding would not pose any problem with this interpretation. However, to test the effect of perturbing the *T-R* equilibrium, a normal citrate agar gel was run in a purged, sealed, nitrogen-containing glove bag. All components were allowed to equibrate for 2 hours. The results were very similar to those obtained in room air, with a possible slight anodal shift in the pattern. These results indicate that the *T* state may make a major contribution to the agaropectin-bound Hb. The presence of excess citrate, which also stabilizes the *T* state, may further help to shift the equilibrium toward the *T* state so that lowering the PO_2 may have little or no effect. Alterations in the pattern in thick gels may be the result of lower PO_2 in the gel center. Similarly, 2,3-diphosphoglyceric acid, inositol hexaphosphate, and other small polyanions should affect the migration. We recently obtained evidence that agaropectin inhibits gelation of Hb S (*14*) and that Hb derivatives which are modified in the region of the β-β cleft have Hb F–like migration (*15*). These observations and the properties of agar gel electrophoresis described above suggest that the anionic polysaccharides constitute a new and interesting class of Hb-interactive agents.

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Very Brief Visual Experience Eliminates Plasticity in the Cat Visual Cortex

Abstract. *Rearing cats in the dark extends the critical period for development of visual cortical neurons, which indicates that the experience of visual input is necessary to begin the developmental process. A single brief pulse of visual input (6 hours) during a period of dark-rearing eliminates delayed development in the visual cortex. Light therefore seems to rapidly trigger the developmental process, and once triggered, that process runs to completion in the absence of further input.*

Postnatal development of the central visual pathways depends on the quality of the visual environment. In cats reared with the lids of one eye sutured closed, the physiology of visual cortical cells is dramatically altered. In normal cats, most cells respond to stimulation of either eye; in monocularly deprived (MD) cats, virtually every cortical cell responds only to stimulation of the initally open eye (*1*). This effect reflects a permanent developmental abnormality and cannot be reversed even by forcing use of the initially closed eye in later life (*1*).

The time period when neural development is susceptible to environmental manipulation is termed the plastic or critical period. It is restricted to very early life. In cats, plasticity appears to be maximal at around 1 month of age and then declines over the next 3 months, after which time the visual pathways are virtually immutable (*2*). During this plastic period, every major response property of cortical cells (ocular dominance, orientation selectivity, direction selectivity, disparity sensitivity) can be modified by manipulation of the visual environment (*3*).

These observations define one of the roles of visual input in cortical plasticity: to determine the final response properties of the developing neurons. In addition to this role, several recent studies have indicated that visual input also plays an essential role in the underlying process of plasticity itself. Animals reared in complete darkness beyond the critical period (until 4 to 10 months of age) and then allowed monocular visual experience show delayed susceptibility to the effects of MD in the visual cortex (*4, 5*). It is as if the critical period has been delayed or slowed in the absence of visual input. These results indicate that the plastic period is not a simple age-dependent maturational process; rather, visual input in some way controls the underlying events that allow for or eliminate neuronal plasticity. The environmental trigger seems to be simply the experience of light. If cats are reared with binocular eyelid suture throughout early life (which allows them to experience only diffuse light), the visual cortex shows dramatic degradation and there is no delayed susceptibility to MD (*5*).

This study was designed to further explore the role of visual input in triggering neural plasticity in visual cortex. We compared visual cortical physiology in cats who experienced prolonged dark rearing with that in cats who experienced very brief visual exposure during prolonged dark-rearing. These two types of cats were compared in electrophysiological studies at the end of the rearing period (to assess development during the restricted visual experience) and after a prolonged period of MD in a normal light-dark cycle following the initial rearing period (to assess the capacity for delayed development and plasticity). We found that very brief periods of visual experience triggered neural development and that once triggered, the plastic period ran its full course in the absence of further visual input.

Nineteen cats were studied. Six received no visual experience throughout the first 4 to 5 months of life [dark-reared (DR)]. Of these, three were used in physiological studies at the completion of the rearing period (DR cats). The other three had the lids of one eye sutured at the end of the rearing period (under ketamine anesthesia, 25 mg/kg) and were allowed prolonged monocular vision prior to physiological study (DR → MD cats). Three cats were reared in darkness for 4 to 5 months, but at the age of 6 weeks they received one day (6 hours) of binocular visual experience in a normally lighted laboratory room. After this rearing, two of them were used in electrophysiological studies [DR(6) cats], then recovered from anesthesia and paralysis and allowed prolonged monocular vision [DR(6) → MD cats]. The third cat simply had one eye sutured after the rearing period [DR(6) → MD]. Four cats were reared in darkness for 4 to 5 months, but at the age of 6 weeks they received two consecutive days (12 hours) of visual experience. Three of these were studied at the end of the rearing period [DR(12) cats], and the other experienced prolonged monocular vision prior to recording [DR(12) → MD cat]. Six other cats were used as comparison animals. In all cats who experienced MD, the period of monocular vision lasted a minimum of 3 months.

Studies of the visual cortex were done with the animals anesthetized (sodium pentobarbital, 25 mg/kg initially, supplemented by 3 mg/kg-hour) and paralyzed (gallamine triethiodide, 10 mg/hour). Recordings were restricted to one hemisphere; in all cats who experienced MD, the hemisphere contralateral to the deprived eye was studied. A minimum of two oblique penetrations (2 mm in extent) were made. Within penetrations, single units were sampled at 100-μm intervals, and all cells studied had receptive fields located 4° to 8° from the area centralis. The same search stimulus (0.5° by 20° white slit) and matched microelectrodes (3 to 5 megohm at 1000 Hz, tungsten) were used in all cats. This procedure ensured representative sampling of ocular dominance clusters within animals and provided objective comparative data among animals. We determined two major receptive field characteristics for each visual cortical cell: ocular dominance (the degree to which the cell responded to visual stimulation of one or