and Table 1), had cells containing metachromatic granules; the cells could not be distinguished morphologically from similar cells in cultures from affected individuals. Gargoyle cells also occurred in small numbers in cultures obtained from both parents (Table 1); because the parents were presumed to be heterozygous for the abnormal gene, the presence of such cells in fibroblast cultures from both parents suggested that the abnormal trait was detectable in the heterozygous state.

Although the fibroblast cultures derived from the father of the patient with the X-linked type of the disease contained no metachromatic cells (Table 1 and Fig. 3*a*), the cultures derived from the mother contained approximately 49 percent of cells with metachromatic granules (Table 1 and Fig. 3*b*). The fibroblast culture from the maternal grandmother of this patient contained approximately 22 percent of metachromatic cells, including some gargoyle cells (Table 1), whereas the culture from the paternal grandmother contained only an occasional metachromatic cell (0.1 percent). Fibroblast cultures from a paternal aunt and a maternal uncle showed negligible metachromasia (Table 1). Proportions of cell populations showing metachromasia remained constant throughout the first 4 months in culture. Other members of this family are now being studied.

Acid mucopolysaccharides stain metachromatically with cationic dyes such as toluidine blue O probably because of the availability of consecutive, regularly spaced anionic groups along the carbohydrate chain (*14*). Although nucleic acids and some acidic lipids of high molecular weight may give a similar but weaker staining reaction, these substances stain purple to violet whereas the sulphated acid mucopolysaccharides stain a strong pink (*15*). It has been shown that fibroblasts from tissues of various origin produce acid mucopolysaccharides in vitro (*16*); and that cultured fibroblasts, regardless of origin, secrete predominantly hyaluronic acid, with only minor secretions of sulphated acid mucopolysaccharides (*17*).

Our observations indicate that, as with cultured lymphocytes (*18*), fibroblasts from patients with Hurler's syndrome have markedly greater contents of acid mucopolysaccharide than those from controls. Moreover, fibroblast cultures from both normal parents of patients having the autosomal recessive form of the disease had detectable metachromatic granules (Fig. 4, *a* and *b*). As to the family of the person having the X-linked type of the disease, the hemizygous state was not demonstrable in vivo but was clearly detectable in vitro. It will be of interest to see whether cloning of the fibroblasts from the mother and maternal grandmother discloses two populations of cells, as has been found in other X-linked genetic traits (*19*).

It is too early to assume that the abnormal gene for Hurler's syndrome can always be detected in cell culture; further studies of more families are required to investigate variation of the expression of the gene under in vitro conditions.

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Response of the Pupil to Steady-State Retinal Illumination: Contribution by Cones

Abstract. *Response of the pupil to steady-state retinal illumination was measured in an observer who lacked functioning rods. At high intensities, this response was as great as that of a normal eye. These results cannot be explained by the hypothesis that only rods are receptors for the steady-state response.*

In 1962 Alpern and Campbell (*1*) summarized the evidence for the idea that the human pupillomotor photoreceptors are both rods and cones. This idea has found general acceptance insofar as responses of the pupil to transient stimuli by light are concerned, but recently Bouma (*2*) questioned it with regard to the steady-state response. Evidence to support the original idea seems overwhelming: (i) steady-state directional sensitivity of the pupillomotor photoreceptors (*3*), (ii) relatively high efficiency of the central retinal area in evoking response by the pupil (*2*), and (iii) continued narrowing of the pupil to increases in light intensity at levels and field sizes that insure that every rod in the retina has been saturated (*2*). Bouma, however, has offered special hypothesis to overcome this evidence, adopting the view that the excitation of rods and of rods alone initiates the steady-state response.

Our experiments demonstrated directly the contribution of cones to the steady-state response; we examined an eye in which exhaustive psychophysical and electrophysiological methods had failed to reveal evidence of functioning rods. This patient (M.L.) has been studied extensively and his color vision, electroretinogram (ERG), electroculogram, and dark-adaptation have been described (*4*). These data together with the results of detailed ophthalmological examination lead to the conclusion that he represents that variety of Oguchi's disease in which no trace of activity by rods appears even after the subject has been in the dark for several hours.

Figure 1*a* shows the change in the absolute visual threshold for a 1.8-degree blue (453-nm) test flash, exposed for 1 second in the nasal field 15 de-

grees from the fixation point for the first 2 hours of dark-adaptation. The solid circles show the results for M.L.; the open circles show the results for a normal eye, with the familiar rod-cone break occurring after about 5 minutes in the dark. For the normal eye the curve for dark-adaptation shows two different parts, each with its characteristic spectral sensitivity. The curve for M.L. has only one part, and measurements for different colors (not shown in Fig. 1*a*) show that its spectral sensitivity is photopic; this suggests that at threshold M.L. has only one kind of functioning receptors—that is, cones. The possibility that he has functioning rods that are greatly reduced in sensitivity and function only above threshold seems to be excluded by the ERG measurements. We could never obtain any corneal-positive *b*-wave (that is, scotopic) responses even under optimal testing conditions (that is, the most intense flash exposed after 30 minutes in the dark). Such stimuli produced only a very small (about 100 μv) corneal-negative *a*-wave, presumably the result of excitation of cones alone.

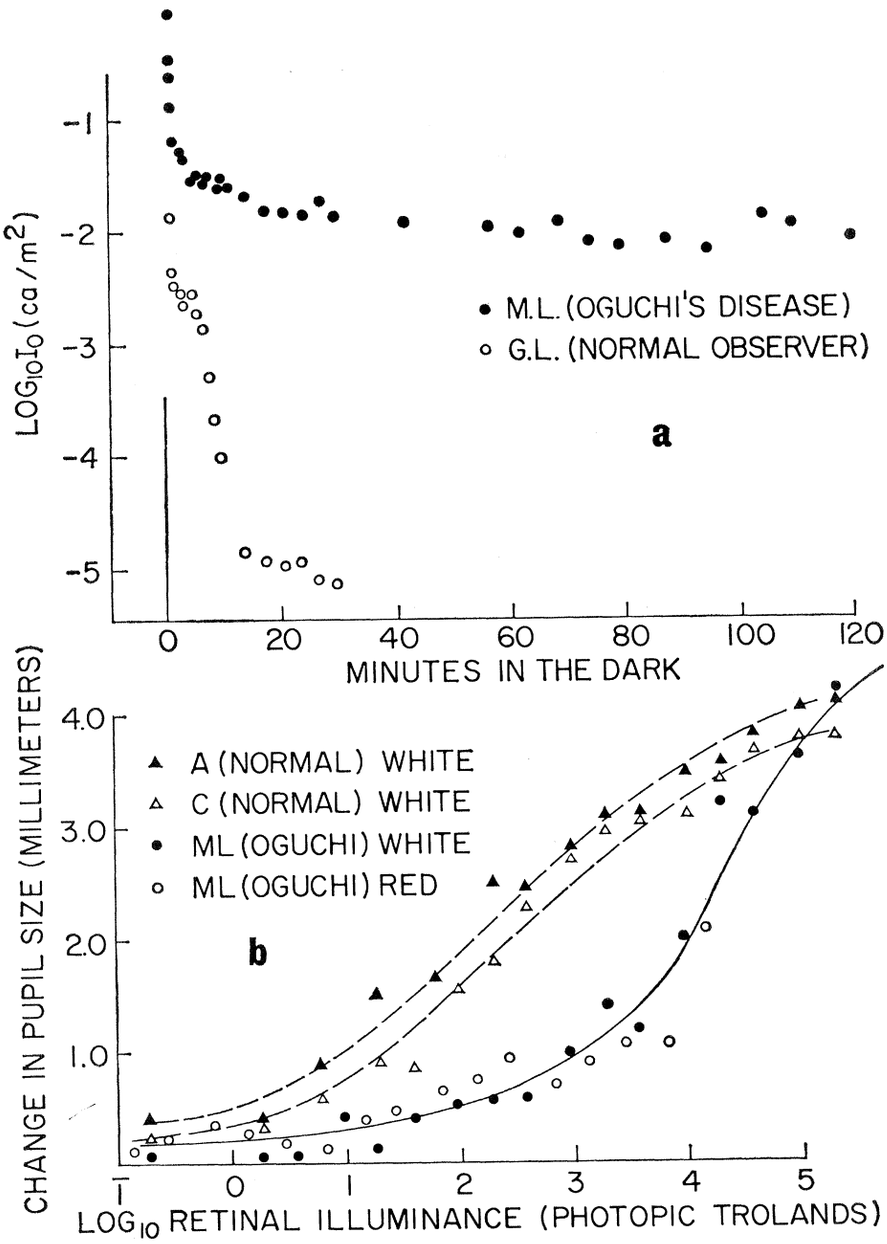

Fig. 1. *a*, Dark-adaptation curves after 10-minute light adaptation to a 30-degree field of 6200 lu/m^2. Test target was 1.8 degrees in diameter, 15 degrees within the nasal visual field of the right eye; dominant wave length, 453 nm. Open circles, normal eye; solid circles, M.L. *b*, Change in diameter of the pupil of the left eye under steady-state illumination of the retina of the right eye. Triangles represent the results for two normal observers, white light; open circles, for M.L. in red light; solid circles, for M.L. in white light. Each point is the mean of 30 measurements.

Response of the pupil of the left eye was studied by infrared photography when the right eye was exposed (under equilibrium or steady-state conditions) to a 12-degree circular test-field seen in Maxwellian view. The image of the tungsten coil in the center of the pupil of the right eye was always smaller than the smallest possible pupil, so that fluctuations of the latter had no influence on the amount of light reaching the retina. The observer was dark-adapted for 30 minutes; he then fixated a small red light, located at infinity, that was just sufficiently bright to be easily seen. Thirty successive photographs were made of the anterior segment of the left eye with a Grass oscilloscope camera, taking about one picture per second. The observer was then light-adapted to the level of the dimmest test field, and after 10 seconds the photographic process was repeated. The process was repeated again at the next lowest level, and in this way the experiment continued until the observer had been adapted to the brightest field intensity. At some intermediate level the field became sufficiently bright so that he could fixate a black spot marked on a clear lantern slide at optical infinity in the stimulus field; once this was possible the fixation light was no longer necessary.

In Fig. 1*b* are plotted the changes in pupil size as a function of the degree of retinal illuminance for two normal subjects (A and C) of about the same age, as well as those for M.L., when the stimulus was "white" light from the tungsten filament. The pupil of M.L. clearly responds to changes of intensity of the steady-state field; indeed, at high but not at intermediate or low levels, the responses of his eye are not obviously different from those of the normal eyes. The possibility that this response may be due to some residual rod activity that was undetected by either the dark-adaptation or the ERG measurements can be excluded by repeating the experiment on M.L. with a red (gelatin) filter (dominant wave length, 625 nm) in the stimulus field. The responses thus obtained are (within the limits of experimental error) superimposed on the responses to white light once the in-

tensities of the red light are corrected for the *photopic* transmission by the red filter. These results seem to lend no support to the view that rods alone serve as receptors for the steady-state "photopupil" response (photopupil refers to narrowing of the pupil in response to stimulation of the retina by light).

We made these measurements by sampling all 30 frames obtained from 10 to 40 seconds (one frame per second) after allowing the eye to be adapted to each new degree of brightness, and not, as Bouma suggests, by selecting only those parts of the response in which the temporal changes of pupil size are minimum. Bouma introduced the hypothesis that his sampling procedure would give results different from those which contradict the "rods alone" theory. His hypothesis would predict for our subject that when the oscillations in the size of pupil were minimal the pupil would dilate to the diameter held by it in total darkness. We tested this prediction by exposing the eye to a bright (4.3 $\log_{10}$troland) field and measuring the size of the pupil every second for 5 minutes. At no time during this 5-minute period did the pupil dilate to within 2 mm of the diameter that it had had in the dark. Moreover, the pupil diameters around which the minimum fluctuations occurred were precisely those values obtained by the sampling procedure used to obtain the measurements in Fig. 1*b* under the same conditions.

No evidence among all the psychophysical, electrophysiological, neuro-ophthalmological, or clinical measurements made by us on M.L. supports the idea that the nerve connections of his retina differ in any way from those of the normal eye. In fact, everything we know about his eyes strongly suggests that his defects are due only to disturbed photopigment kinetics in his rods and cones. Thus, we have clear evidence that in an eye lacking functioning rods there can be quite unequivocal steady-state photopupil responses. We infer that in a normal retina both rods and cones serve as photoreceptors for steady-state photopupil response in the same way that they serve for the transient photopupil response.

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Primary Immune Reactions in Organ Cultures

Abstract. *Primary immune reactions were initiated and maintained in vitro. Spleen explants from mice treated with either phytohemagglutinin or adjuvant were stimulated to form hemagglutinins and hemolysins, whereas no treatment of spleen donors was necessary to invoke a "graft-versus-host" response which led to specific splenomegaly.*

Interest in the mechanism of antibody formation has led to repeated attempts to induce primary immune responses in vitro (*1*). Probably two types of cells are involved, macrophages and lymphocytes, and an interaction between these two cell types may be essential in the development of an immune response (*2*). However, in previous experiments both the yield of antibodies and the frequency of positive cultures were quite low (*2*). A possible explanation for the difficulties encountered in the initiation of immune reactions in vitro was the fact that lymphocytes fail to proliferate under the usual conditions of culturing, while lymphoid proliferation may be important in the early stages of antibody formation. Furthermore, the tissue culture methods used in previous studies were not designed to encourage cellular differentiation, which may also be a key element in the initiation of immune reactions.

In our experiments an organ culture method was used which permits lymphoid maintenance, differentiation, and proliferation (*3*). Spleen fragments, 0.2 to 0.3 mm in thickness and 0.5 to 1.0 mm^2 in surface area, were explanted in the well of a Millipore filter assembly prepared by gluing a TH Millipore filter (porosity 0.45 μ, thickness 25 μ) to the underside of a plastic mount (*4*). The assembly was placed in an organ culture dish (Falcon 3020) containing 1.0 ml of a tissue culture medium consisting of Eagle's basal medium supplemented with 10 percent of horse serum (Difco 0569; or A gamma-globulin, Hyland 117-100, for studies of agglutinins), 5 percent chick-embryo extract (9-day embryos), erythromycin (50 μg/ml), and mycostatin (50 μg/ml). Cultures were incubated at 37.5°C in a water-saturated atmosphere of 95 percent oxygen and 5 percent carbon dioxide. Mice of strains Balb/cHeAu, C_3H/HeAu and F_1 ($C \times C_3H$) were used in all experiments.

In "graft-versus-host" reactions embryonic intact spleens from 17-day embryos and spleen fragments from 1- to 4-day-old F_1 ($C \times C_3H$) mice were used as host tissues. Cultures were set up in pairs, and the size of explants was carefully matched for each pair. Spleen cell suspensions were prepared from C_3H (parental) or F_1 ($C \times C_3H$) (syngeneic) adult mice by passing minced tissue through syringe needles of progressively smaller bore. Approximately 5×10^5 nucleated cells were added to the explanted "host" spleens, and cultures were scored after 3 to 4 days. Explants grown in the presence of parental cells were compared to explants grown in the presence of syngeneic cells (40 cases) or to explants grown without any cells added (25 cases). The presence of parental cells led to marked splenomegaly (1.5- to 3-fold enlargement) in all 65 cases (Fig. 1*a*). The histological changes occurring within the explanted spleen tissues were similar to those in vivo in typical graft-versus-host reactions (*5*). The sequence of histological changes included lymphoid cell accumulation and dispersion, increased mitotic activity associated with active granulocytopoiesis, and ultimate lymphoid-cell degeneration (*6*). Experiments with allogeneic as well as parental cells indicate that immunological maturation of the spleen occurs within 3 days after birth, at which time a typical "discriminant spleen assay" (*7*) can be obtained in vitro.

To stimulate antibodies to sheep red blood cells, spleen fragments were obtained from adult C_3H mice which were free of natural agglutinins as concluded from tests performed on serum samples. The sheep blood cells (Gibco)