had been eluted from the pellets. This rapid exposure of the bladder to saccharin is comparable to that observed for sodium cyclamate (*5*) and the 8-methyl ether of xanthurenic acid (*13*), compounds demonstrated to possess significant carcinogenic activity for the mouse bladder when suspended in cholesterol and tested by the pellet implantation technique (*5*, *8*).

The survival of mice for more than 175 days after surgery, the average length of survival, and the incidences of squamous metaplasia and carcinoma in the bladders were compared in the duplicate test and control groups of mice (Table 1). The first bladder carcinoma was observed in a mouse treated with saccharin that died 293 days after surgery. Statistical comparisons in both experiments demonstrated that mice exposed to sodium saccharin exhibited significantly higher incidences of bladder carcinomas (47 and 52 percent) than did the controls (13 and 12 percent). The carcinomas in mice exposed to saccharin were often visualized on gross examination, were frequently multiple within the same bladder, were more often invasive into muscle ($P = .009$), and were often composed of cells demonstrating high degrees of mitotic activity and pleomorphism (Fig. 2). Squamous and glandular metaplastic epithelial alterations were more frequent in mice exposed to saccharin. No benign papilloma was seen in the bladders of saccharin-treated or control mice. No other tissues of mice exposed to sodium saccharin demonstrated a tumor incidence significantly different from that of the control groups of mice.

The degree of histologic malignancy (Fig. 2) observed in the bladder tumors produced by saccharin was more severe than that seen in mouse bladder carcinomas produced by sodium cyclamate (*5*). Moreover, the bladder carcinomas produced by saccharin were as malignant in appearance as those induced in mice, rats, and dogs (see *14*) by feeding the potent urinary bladder carcinogen *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide, and as severe as those obtained by testing *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (*15*) or other carcinogens (*8*, *13*) by the mouse bladder pellet implantation technique.

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References and Notes

1. C. Fahlberg and I. Remsen, *Chem. Ber.* **12**, 470 (1879).
2. M. E. Rubini, *Amer. J. Clin. Nutrition* **22**, 229 (1969).
3. O. G. Fitzhugh, A. A. Nelson, J. P. Frawly, *J. Amer. Pharm. Ass.* **40**, 583 (1951).
4. M. J. Allen, E. Boyland, C. E. Dukes, E. S. Horning, J. G. Watson, *Brit. J. Cancer* **11**, 212 (1957).
5. G. T. Bryan and E. Ertürk, *Science* **167**, 996 (1970).
6. J. M. Price, C. G. Biava, B. L. Oser, E. E. Vogin, J. Steinfeld, H. L. Ley, *ibid.*, p. 1131.
7. J. J. Schrogie, *Food Drug Admin. Papers* **3** No. 9, 28 (1969).
8. G. T. Bryan, *Amer. Ind. Hyg. Ass. J.* **30**, 27 (1969); *J. Nat. Cancer Inst.* **43**, 255 (1969).
9. Obtained as a gift of analytically chemically pure sodium saccharin (Lot No. 3938-01) from Abbott Laboratories, North Chicago, Ill.
10. G. T. Bryan, R. R. Brown, C. R. Morris, J. M. Price, *Cancer Res.* **24**, 586 (1964).
11. G. M. Bonser and J. W. Jull, *J. Pathol. Bacteriol.* **72**, 489 (1956); F. J. C. Roe, *Brit. J. Urol.* **34**, 238 (1964).
12. R. A. Fisher, *Statistical Methods for Research Workers* (Hafner, New York, ed. 13, 1958), pp. 96–97.
13. G. T. Bryan, R. R. Brown, J. M. Price, *Cancer Res.* **24**, 596 (1964); G. T. Bryan and P. D. Springberg, *ibid.* **26**, 105 (1966).
14. E. Ertürk, J. M. Price, J. E. Morris, S. M. Cohen, R. S. Leith, A. M. Von Esch, A. J. Crovetti, *ibid.* **27**, 1998 (1967); E. Ertürk, J. M. Price, G. T. Bryan, *Proc. Amer. Ass. Cancer Res.* **10**, 23 (1969); E. Ertürk, S. M. Cohen, J. M. Price, G. T. Bryan, *Cancer Res.* **29**, 2219 (1969); E. Ertürk, S. M. Cohen, G. T. Bryan, *ibid.*, in press; E. Ertürk, S. M. Cohen, O. Yoshida, J. M. Price, G. T. Bryan, *Abstr. 10th Intern. Cancer Congr.*, in press.
15. E. Ertürk, O. Yoshida, G. T. Bryan, in preparation.
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Testing the Vision of Cataract Patients by Means of Laser-Generated Interference Fringes

Abstract. *In a new technique for measuring the visual acuity of cataract patients the light from a laser is used to form interference patterns of variable fineness on the patient's retina. The fineness of the interference pattern that the patient can detect gives an indication of the potential for improved vision. Comparison of this estimate of the potential with the patient's vision after cataract extraction shows that this test can indicate the condition of the fovea behind a cataract.*

A cataract, which is a loss of lens transparency, most often occurs in persons past middle age and appears to be part of the general aging process. In addition, cataracts can occur secondary to ocular or systemic disease and from traumatic or perforating injury to the globe. A developing cataract causes a gradual and painless loss of vision. When the cataract has progressed to the point where the reduction in vision remaining after the correction of refractive error interferes with normal activities, the only treatment is surgical removal of the lens. Good vision may be surgically restored if changes in the fovea have not occurred prior to surgery. The lens opacities often make it difficult to detect macular changes by ophthalmoscopic examination. The usual methods of preoperative testing such as discrimination between two point images and color perception may be poor indicators of the functional integrity of the fovea centralis.

Campbell and Green (*1*) have produced high-luminance interference fringes on the retina with a low-power gas laser. These fringes are not degraded by ordinary optical aberrations. If one views the fringes with a piece of tissue paper or milk glass held over the eye, one sees the fringe pattern disturbed (see cover). However, it is still possible to resolve fine stripes corresponding to the equivalent of 20/20 visual acuity because microscopic holes in these materials allow portions of the focused beams to pass unscattered. If an interferometric technique could produce fine, high-contrast fringes on the retina behind a cataractous lens, it would make it possible to determine the condition of the fovea. This report presents the results of a series of experiments on patients with cataracts to determine the feasibility of preoperatively testing the visual potential by means of laser-generated interference fringes.

The method used to produce interference fringes on the retina will be briefly described here; the full details are available elsewhere (*1*, *2*). The output from a low-power, helium-neon gas laser was optically divided into two parts. A lens projected an image of the doubled laser source into the eye of the patient. This can be done with safety because the total power of the light entering the eye is less than 0.1 mw. In addition, the light is dispersed over a large area so that the amount of light falling on the retina is no greater than that of ordinary daylight illumination. The spacing between the two point images determines the fineness of the pattern formed on the retina. To increase the likelihood of finding a relatively clear optical path for the coherent light through the cataract, a mydriatic was used to dilate the pupil of the patient's eye.

The patient's head was restrained by a headrest, and a system of mechanical slides allowed the double images to be moved in the dilated pupil to those regions of the cataract that were relatively transparent. If the referring physician's clinical description of the cataract did not indicate the location of a less dense region of the cataract, the patient's reports of subjective brightness were used to assess relative density.

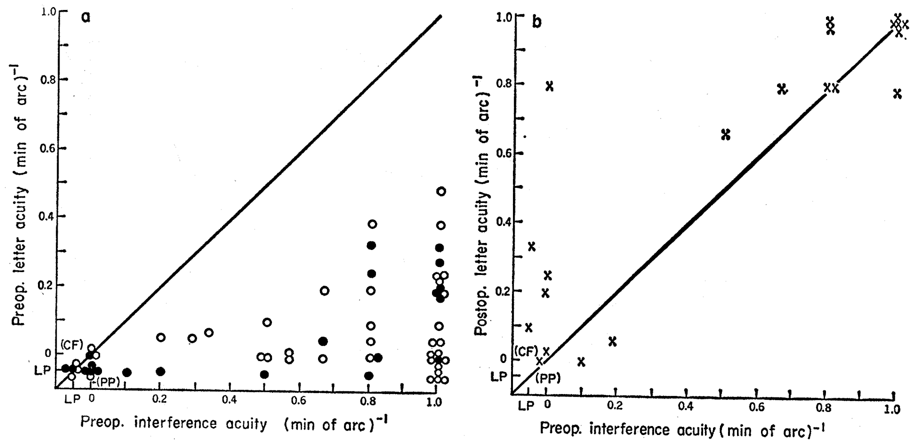

Fig. 1. (a) Comparison of preoperative letter and preoperative interference fringe acuity (circles) of patients whose eyes have cataractous lenses. A filled circle is used if a letter acuity determined after surgical removal of the lens is plotted in Fig. 1b. (b) Relationship between postoperative letter acuity and preoperative interference fringe acuity (crosses). CF, "counts fingers" vision.

Patients often first reported seeing moving random patterns of bright and dark irregular shapes. They reported that this "jumble" appeared to be in front of the striped pattern of interference fringes. In order to see the fringes the patient had to learn to ignore the changing and disordered patterns. If the cataract was unilateral or if sufficient vision still remained, the patient was shown photographs of fringes taken with tissue paper or milk glass placed in front of a camera. This seemed to aid the patient in learning to detect the interference fringes.

The test was started with coarse fringe patterns. The gratings were presented at random in horizontal or vertical orientation. When the patient reported seeing the stripes, he was asked to report their orientation. If the orientation of the fringes could be correctly identified, it was assumed that the patient was able to see the pattern. Even if the coarse stripes were not seen, the spacing between the double images was gradually increased, thus making the patterns progressively finer. Sometimes a patient who was unable to see the coarse fringes would report seeing the finer striped patterns. Several times either the jumble was confused with a striped pattern or the visual task was not correctly understood and patients would report seeing stripes but were unable to correctly identify their orientation.

Figure 1 summarizes the results of tests on 61 cataractous eyes in 50 individuals. Grating and letter acuities have been related by assuming that when the angular subtense of the strokes in the letter equals the width of a single bar in the array of parallel alternating dark and light fringes, both targets can be equally well resolved (*3*). Visual acuity is expressed as the reciprocal of the smallest resolvable angle in minutes of arc and is equal to the fractional acuity, as commonly measured clinically, in decimal equivalent.

The circles shown in Fig. 1a relate preoperative letter acuity to preoperative interference fringe acuity. If there were perfect correlation between the two measures of acuity, all of the circles should fall on the line of unit slope shown in Fig. 1a. The extent to which the circles fall to the right of the line indicates increased acuity in the laser fringe test. In particular, if an acuity of 0.67 (20/30) or better is arbitrarily defined as "good" vision and an acuity of 0.25 (20/80) or worse is designated as "poor" vision, then 30 individuals (36 eyes, 60 percent of the total number of eyes examined) in the preoperative group indicated a potential for good vision. For those individuals with a potential for good vision the preoperative letter acuities ranged from 0.5 (20/40) to the ability to perceive light. Over half of these had a letter acuity of 0.1 (20/200) or worse. On the other hand, 12 individuals (13 eyes, 21 percent of the total) were unable to see the fringes. These individuals have been divided into two categories, those whose eyes are capable of light perception (LP) and those whose eyes are capable of pattern perception (PP), depending on whether the patients reported seeing the disordered pattern or not.

There can be little question but that individuals who achieve a fringe acuity of 1.0 (20/20) have a potential for good vision. What remains to be determined, however, is whether the gradations in preoperatively measured fringe acuity correlate with the potential for improved vision. Postoperative letter acuities (*4*) are plotted (crosses, Fig. 1b) against preoperative fringe acuities, for the 18 individuals (21 eyes) who have had one or both lenses surgically removed. The extent to which the crosses cluster around the line of unit slope indicates the correlation between the predicted and actually achieved acuities. Nine individuals (11 eyes) in the operated group indicated a potential for good vision and eight individuals (nine eyes) either indicated poor potential or could not see the fringes. Postoperatively, all nine individuals (11 eyes) who could see the fine fringes now have good vision. Out of the eight individuals (nine eyes) for whom the test either indicated poor potential or was inconclusive, two individuals (two eyes) have better than poor vision. Moreover, five individuals (five of the eyes in this group) have acuities of 0.1 (20/200) or less and these individuals are therefore legally blind.

Although it is not clear how the laser light penetrates through a cataract, there are at least two possible explanations. First, since the patterns are not imaged onto the retina but rather are formed by the interference of light from two pinpoint sources focused into the lens, it seems that sufficient amounts of light could enter the eye unscattered through microscopic holes even when the whole lens appeared opaque. Second, the interference technique permits one to bypass the effects of optical aberrations and thereby to effectively take advantage of any clear areas remaining in the lens. In addition, the red light from the helium-neon laser is probably scattered less than light of shorter wavelengths is. The above factors permit the principal causes of poor vision, the scattering of light from the cataractous parts of the lens and the poor image-forming properties of the peripheral parts of the eye's optics (*5*), partially to be circumvented.

In any event, in order to produce regular patterns on the retina behind an opaque lens, at least some portion of the coherent light must pass through the lens unscattered. It seems that the denser the cataract, the more difficult it becomes for patients to detect the regular fringes in the disordered pattern produced by the cataract. Particularly if the patient has a dense cataract, failure to see the fringes may not necessarily indicate a lack of potential for good vision.

The results reported here indicate that preoperatively determined interference fringe acuity can be used to assess the potential for visual improvement in a significant number of cataract patients. Furthermore, it seems likely that this test can be used to differentiate between retinal and optical causes of visual loss in a host of other disorders involving the dioptrical apparatus of the eye.

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References and Notes

1. F. W. Campbell and D. G. Green, *J. Physiol. London* **181**, 576 (1965).
2. F. W. Campbell, J. J. Kulikowski, J. Levinson, *ibid.* **187**, 427 (1966); D. E. Mitchell, R. D. Freeman, G. Westheimer, *J. Opt. Soc. Amer.* **57**, 246 (1967).
3. One might think that it is impossible to relate the resolution of such complex patterns as letters to that of gratings in a simple way. However, the literature [for example, Y. Le Grand, *Form and Space Vision* (Indiana Univ. Press, Bloomington, 1968)] indicates that for the normal eye there are only small differences at photopic luminances between grating and letter acuity. Does the generalization that grating and letter acuities are equal (when the acuity is expressed in terms of the angle subtended by the smallest detail) hold for patients with less than normal vision? In an effort to answer this question, letter and fringe acuities were measured on a group of 19 patients (23 eyes) having various degrees of visual loss caused by other than optical factors. These patients had a mean letter acuity of 0.38 and a mean interference fringe acuity of 0.42. The coefficient of correlation between letter and grating acuity was .95.
4. The letter visual acuities were determined by the patient's physician, using suitable lenses to correct for refractive errors. Generally, the postoperative acuity was obtained 3 or more months after surgery when it seemed likely that the eye had achieved its full potential for improvement.
5. D. G. Green, *J. Physiol. London* **190**, 583 (1967).
6. Supported in part by PHS grant EY 379.

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Circadian Rhythm of Brain Self-Stimulation Behavior

Abstract. *Under constant conditions of light, sound, temperature, and humidity, rats exhibited circadian rhythmicity in rate of bar-pressing with hypothalamic and septal reinforcing brain stimulation. Variations in reinforcer magnitude affected absolute levels of operant response emission but not the frequency of the circadian rhythm. In long sessions, the time of peak responding deviated systematically from a strict 24-hour period. Such data show marked similarity to free-running rhythms of motor activity.*

Oscillation is a temporal property that unites and interrelates biological systems. Studies of behavioral oscillation have concentrated on stabilimetric activity or locomotor response chains. Both of these measures reflect complex and unspecified behavioral repertories, and various subsets of such behavior samples may dominate an activity count at different times. Overall rates of behavior output do not change as much as the relative dominance of particular topographical units (*1*). Isolation and measurement of a narrowly defined, highly probable response unit would likely lead to a more refined analysis of cyclic behavior patterns and their associated physiological oscillation mechanisms. A popular way to insure the high probability of a response is to reinforce it, rather than to rely on its "spontaneous" emission. For our experiments, we selected the relatively stereotyped operant bar-press as the unit for measurement. To maintain responding for long periods, we used brain stimulation as the reinforcer, eliminating (or minimizing) the rapid satiation effects found with traditional nutritive reinforcers. We report a circadian periodicity in operant response rate during long-term self-stimulation sessions, with the use of a variety of stimulation parameters, electrode loci, and reinforcement schedules. The cyclic response patterns show many of the same characteristics as locomotor activity as well as hormonal, neural, and cellular rhythms, and may reflect common biological oscillation mechanisms (*2*).

Male albino laboratory rats (Charles River, CD strain) were implanted with septal or hypothalamic bipolar electrodes (Plastic Products, MS 303), or both, and were allowed to self-stimulate in continuous sessions for as long as 1 month. Placements were verified by subsequent histological analysis. Bar-pressing was reinforced by 0.5-second trains of constant-current bidirectional rectangular pulses, 0.2 msec in duration, equally spaced in time. A single peak-current level and electrode locus were chosen for a given session. The experimental compartment was enclosed in a controlled environment chamber (Sherer-Gillett, CEL 44) with dry food and water continuously available. An incandescent lamp illuminated the chamber, maintaining a constant luminance of 10 ft lam (10.7 mlam) on the wall at the level of the bar. External noise was attenuated by the multilayered enclosure, and temperature was set at 70°F (21°C) with 50 percent relative humidity (*3*).

When an animal starts a self-stimulation session, it usually responds at a high rate without resting. Subsequent deceleration in response rate occurs, but its onset depends on stimulation intensity. At high intensities the initial response spurt may last as long as 2 days without rest periods of more than a few minutes. Only later does the animal periodically slow its responding and take long enough rests to exhibit a circadian rhythm. Initial response deceleration, occurring after about 8 hours of self-stimulation (sometimes thought to parallel a food "satiation" effect) has previously been reported only for septal placements (*4*). We observed the effect for both hypothalamic and septal loci with low and medium stimulation intensities; at high intensities the initial response deceleration was greatly delayed.

Figure 1 includes continuous week-long samples of response output from several animals under a selection of locus, intensity, and schedule conditions. Responses were tallied in 6-hour blocks, and smooth curves were fitted by eye. The records show wide differences in peak rates under the various reinforcement conditions (ranging from approximately 500 to 3700 responses in a 6-hour block), as well as in day-to-