that periodic torpor (hibernation or aestivation) occurs in this and other species of the genus *Perognathus* as a response to low ambient temperature or absence of food. Tucker (*5*) reported torpor can occur daily in *P. californicus* when food supply is reduced. The amount of time in torpor varied inversely with the quantity of food available. Animals in the laboratory may undergo periodic torpor even with an abundance of food and normal ambient temperature (*6*).

During the period of our radiation dose measurements wild *P. formosus* were predominantly inactive and hence apparently torpid only when environmental temperatures were low. There was no indication of a shortage of food, even late in the year. The lengthening periods of activity during successive months in spring may be a response to increasing average temperatures or to increasing food supply. Since metabolic rate in these animals varies greatly with activity, two similar populations in two different years may have vastly different energy requirements.

The labile body temperature and metabolic rate in pocket mice is of great importance to survival in an environment characterized by unpredictable growing seasons, by prolonged drought, and by long periods of high temperatures. All members of the genus *Perognathus* that have been investigated respond similarly to changing environmental conditions, and these results may be relevant to the observed survival of another species. Our trapping records revealed surprisingly long survival times for the smallest species, *P. longimembris*, which as adults weigh only 6 to 8 grams. Twenty-five marked individuals survived between 3 and 5 years over a period of particularly severe environmental conditions. These observations suggest that increasing periods of torpor may prolong the life span, although the total activity time of these animals may remain unchanged.

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References and Notes

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7. Studies supported by contract AT (04-1) GEN-12 between the U.S. Atomic Energy Commission and the University of California. Dosimeters were made and read by Edgerton, Germeshausen & Grier, Inc., Santa Barbara, California.

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Holomicrography: Transformation of Image during Reconstruction a posteriori

Abstract. *Holomicrographs recorded through a microscope contain a hologram of the interior of the microscope, including the objective. During reconstruction of the microscopic image, modification of the aperture of the reconstructed objective produces the same alteration in the reconstructed image that would have occurred in the original image, if the actual objective had received the same modification. By this means, a single event holographed by bright-field microscopy may later be examined in reconstruction by dark-field, phase-contrast, or interference microscopy.*

A diatom, *Navicula lyra* Ehr., was holomicrographed (*1*) in light (wavelength, 6328 Å) from a Spectra-Physics model 125 helium-neon c.w. gas laser. The unmodified beam from the laser was passed through a beam splitter, reflected from a first-surface mirror through an objective, [10 ×, numerical aperture (N.A.) 0.30] used as a condenser, through the specimen, and through another objective (40 × N.A. 0.65) to the film plane of a 35-mm camera body, holding Eastman Kodak 649-F spectrographic film. The reference beam arising at the beam splitter was reflected obliquely from a first-surface mirror, passed through a 20 × objective at about the level of the view-

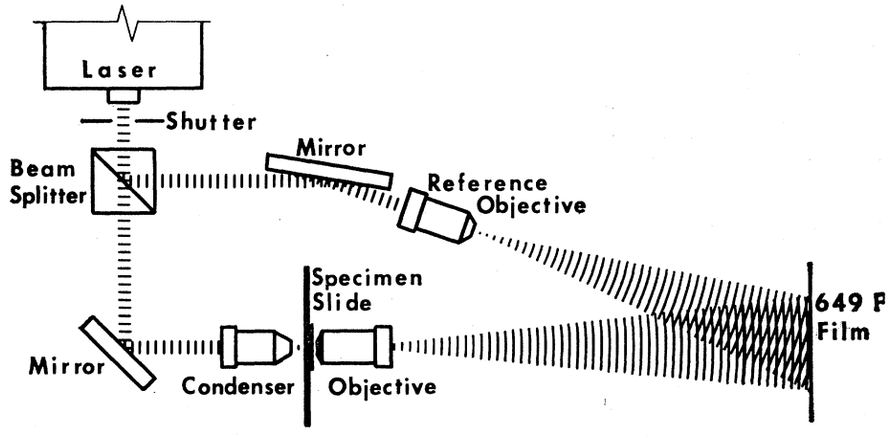

Fig. 1. Diagram of holomicrographic recording system in plan view.

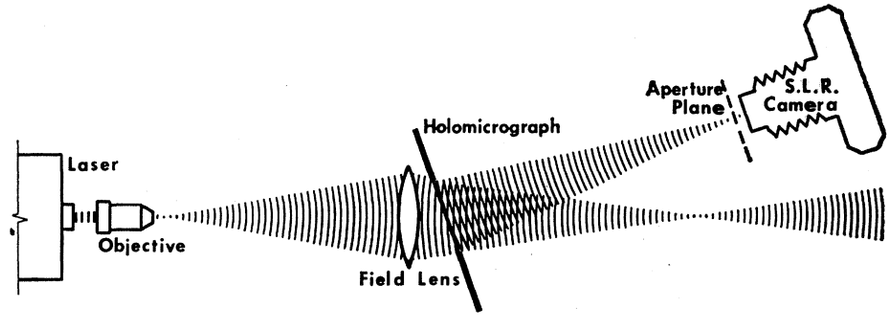

Fig. 2. Plan view of apparatus arrangement for photographing reconstructed images. The aperture plane designated is that of the reconstructed objective. With this aperture unmodified, the reconstructed image photographed is similar in all significant aspects to the original image available in the microscope at the time the hologram was recorded. Modification of this aperture as described in the text and Fig. 3 transforms the image.

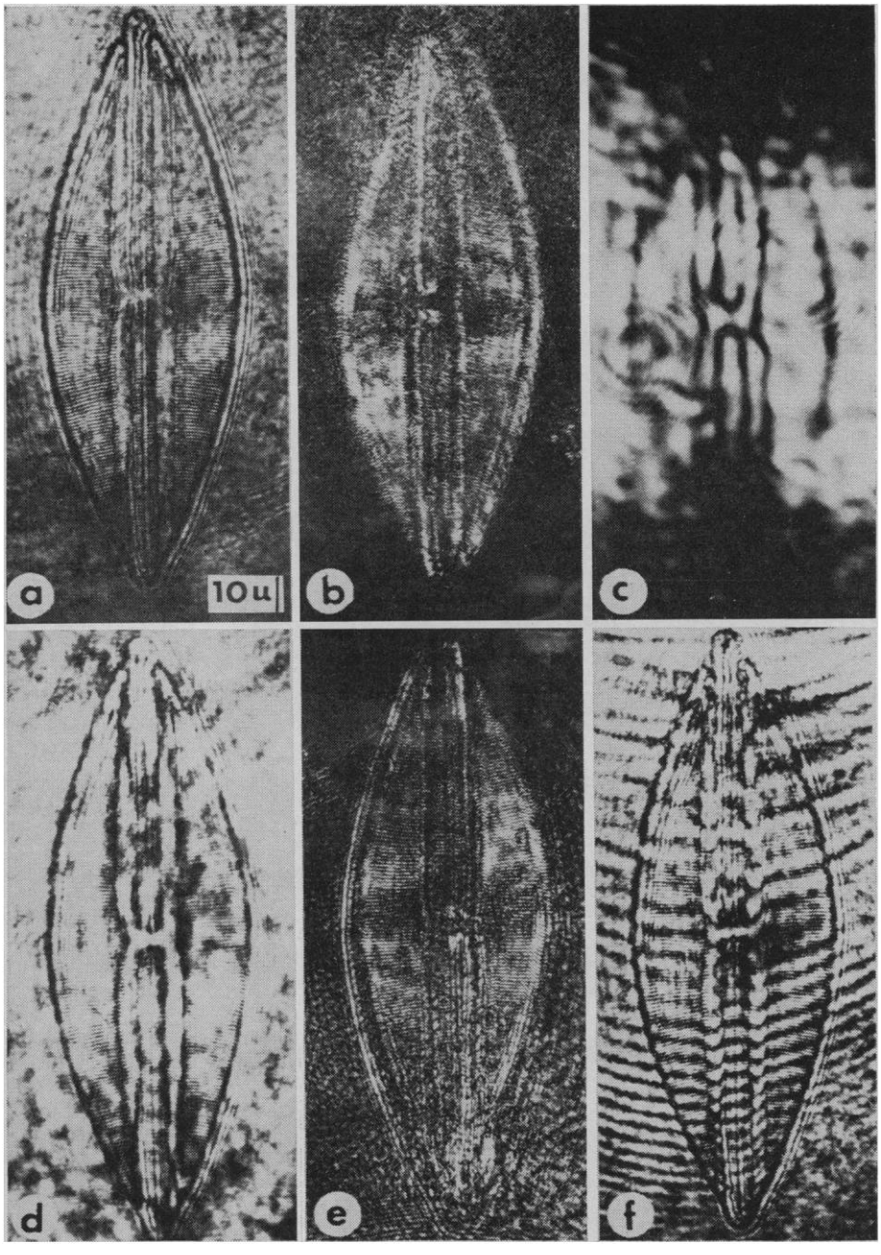

Fig. 3. Virtual image of a diatom, *Navicula lyra*, reconstructed from the same holomicrograph at the same focus. (a) Unmodified reconstruction. The image is a faithful reproduction of the original microscope image and shows the resolution expected from the 0.65 N.A. objective illuminated with 6328 Å coherent light. Spatial filtering of the illuminating beam, not used in this experiment, would eliminate some of the background noise. (b) Dark-field reconstruction. This image is formed by blocking the direct light at the reconstructed objective aperture. (c) Spatial filtering. The fine image detail has been removed by reducing the aperture of the reconstructed objective. For the example shown, the objective image was masked with a heavily sooted glass plate with a small clear aperture to pass the direct light. This figure shows that the aperture used was not exactly coincident with the objective aperture, that it was not circular, that it contained loose soot particles and that a simple pinhole would have been a more effective and logical choice. (d) Bright-ground positive phase contrast. For this figure a lightly sooted glass plate with a small clear aperture was used as a crude phase-retardation and absorption plate to mask the reconstructed objective aperture. (e) Dark-ground negative phase contrast. A small partially absorbing soot spot attenuated and retarded the direct light at the reconstructed objective aperture. (f) Interference fringes. A small lenticular wedge located at the reconstructed objective aperture divided the direct light into two beams to produce the fringes at the image plane. The fringe deviations produced by the specimen image are similar to those produced by the original specimen observed with a Lietz interferometer microscope.

ing objective, and impinged on the plane of the film at an angle of about 19° to the specimen beam (Fig. 1). The recording technique derives from the Leith and Upatnieks (*2*) carrier-wave method of holography and is somewhat simplified from van Ligten's (*3*) technique of holomicrography. Recoil from the shutter in the camera body tends to destroy the carrier recording; best results were obtained with exposures of ¼ to ½ second (*4*) through a simple shutter, independently supported and interposed between the laser and the beam splitter.

For reconstruction the laser beam was focused by a 10×, 0.25 N.A. objective, and the point of focus was projected on the axis by a 7-inch (18-cm) *f*2.5 field lens at about 1:1 magnification. The holomicrogram was placed close to the field lens and viewed at about 19° to the illuminating axis. Looking through the hologram as a window, one sees—from one side of the illuminating axis—the inside of the camera body, as viewed from the film plane, and a virtual image of the microscopic specimen; toward the observer from the hologram is a real image of the objective aperture. The view from the other side of the illuminating axis, or from the same side with the holomicrograph reverted right to left, reveals a real image of the specimen and of the inside of the camera (turned inside out); and on the far side of the hologram there is a virtual image of the objective.

Viewing the virtual image of the specimen through a long-focus lens on a single-lens reflex camera, with the lens just distal to the aerial image of the microscope objective, sets the conditions for image conversion (see Fig. 2).

With the system arranged as described, the aerial image of the objective aperture may be modified, and the resulting image may be photographed. Figure 3a shows the unmodified image reconstructed from the holograph. Figure 3b shows the "central-stop" dark-field image that results from blocking the zero order or direct light at the aperture image while passing the diffracted light. Figure 3c shows the spatial filtering that results from blocking most of the diffracted light by passing the direct light through a mask that was approximately the reciprocal of the dark-field mask. Figure 3d shows a crude form of phase-contrast image produced by

passing the zero order through a cleared spot on a lightly sooted plate. The soot layer served to retard and partially absorb the diffracted light with respect to the zero order resulting in bright-ground positive phase contrast. Figure 3e shows the dark-ground negative phase contrast image that results from passing the direct light through an absorbing and retarded layer while leaving the diffracted light unchanged. Figure 3f results from passing part of the direct light through a shallow lenticular wedge and illustrates a simple form of interference microscopy. This figure clearly illustrates that both phase and amplitude information are present in the reconstructed image.

Although not specifically tested in this study, it is obvious from the results shown, particularly Fig. 3f, that conventional double-beam interferometry could be performed during reconstruction as Horman (*5*) has suggested. Furthermore, Lohman's (*6*) proposal for the use of an additional reference beam, polarized normal to the first, would permit recording the polarization state of the wavefront to make possible retroactive polarization microscopy.

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References and Notes

1. In current usage photomicrography is photography of the magnified image produced by a microscope, while microphotography is the production of very tiny photographs. By analogy I have used the term holomicrography to designate holography of magnified microscope images.
2. E. Leith and J. Upatnieks, *J. Opt. Soc. Amer.* **53**, 1377 (1963).
3. Discussed in a paper by H. Osterberg and R. van Ligten presented at the seventh session of the International Commission of Optics during the Congress on Recent Progress in Optical Physics in May 1966 in Paris. An abstract of this report is in *Laser Focus* **2**, No. 11, 7 (1966).
4. Kodak 649-F film can resolve more than 1500 lines per millimeter and has a relative speed rating of ASA 0.025. At a 19° angle used between specimen and reference beams, the resolution required of the film is about 500 lines per millimeter. Kodak Special High Definition Aerial Film provides this resolution at a 64-fold increase in speed. My system of illumination did not make the most efficient use of the light available, but at the outside, the energy required for the exposure was about 17 mjoule with less than half of this passing through the field of view. Reducing this to ¼ mjoule for the increase in emulsion speed would place 1-msec exposure time well within the range of feasibility with currently available continuous wave or pulsed ion lasers.
5. M. H. Horman, *Appl. Opt.* **4**, 333 (1965).
6. A. W. Lohman, *ibid.*, p. 1677.
7. Supported by USPHS grant CA04552-08 to Shinya Inoué; equipment purchased under NSF grant GB2060 to Dr. Inoué and NSF grant GB2028 to the author.
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Evidence from Cultured Leucocytes of Blood Cell Chimerism in Ex-Parabiotic Frogs

Abstract. *Postmetamorphic diploid and triploid frogs that had earlier been joined in parabiotic union from embryonic life until metamorphosis were each found to be chimeric with respect to their blood cells, as revealed in chromosome preparations of cultured leucocytes. Blood cells precursors most likely were interchanged when the ex-parabionts shared a common circulation in embryonic life, and the exchanged precursor cells apparently homed in the hematopoietic tissues of the hosts. The tolerance which exparabiotic pairs of frogs exhibit toward grafts of each other's skin is attributable to the blood cell chimerism.*

Owen's far-reaching finding two decades ago (*1*) that dizygotic, or two egg, cattle twins are genetically chimeric with respect to their red blood cells is now a familiar fact. Each member of a pair possesses not only its own antigenically distinct kind of erythrocyte, but also the antigenic type of its twin. The erythrocyte chimerism is traceable back to events in fetal life. The anastomosis of placental vessels permits the reciprocal exchange of primordial blood cells, and the translocated embryonal blood-forming cells establish themselves in the hematopoietic tissues of the hosts. The erythrocyte chimerism is accompanied by a state of specific immunologic unresponsiveness, or tolerance. A chimeric calf accepts homografts of skin from its co-twin (*2*), although the tolerance to skin homografts may not necessarily be permanent (*3*).

The natural twinning in cattle can be duplicated experimentally in organisms that lend themselves readily to microsurgical manipulations in early development. One such organism is the leopard frog, *Rana pipiens*, the subject of many classical studies in experimental embryology. In recent years, interest in embryonic tissue transplantation and embryonal parabiosis in the frog has been renewed from the standpoint of modern immunological concepts (*4*, *5*). Embryos of the leopard frog joined in parabiosis become separated from each other during their metamorphosis into juvenile frogs. Postmetamorphic ex-parabiotic frogs are highly tolerant of skin grafts from their former partners (*5*).

The mutual acceptance of skin homografts by ex-parabiotic pairs of frogs renders it likely that the copartners are blood cell chimeras. Heretofore, this has remained more an article of faith than actual demonstration. The purpose of this report is to present evidence that the ex-parabiotic frogs do contain a mixed population of blood cells.

The problem was approached by bringing into play two experimental procedures that are ideally applicable to the leopard frog, namely, the induction of triploidy and the in vitro cultivation of leucocytes of peripheral blood. Triploidy was induced by pressure treatment, after the method of Dasgupta (*6*). We support Dasgupta's findings of a high incidence of triploidy (as high as 85 percent) when the eggs, 5 minutes after insemination, are subjected to hydrostatic pressure of 5000 lb/in.^{2} (350 kg/cm^2) for 6 minutes. A triploid embryo is distinguishable in early development on the basis of size and spacing of lightly pigmented cells of the head ectoderm (*7*).

Embryos were joined in parabiosis at the tail bud stage of development

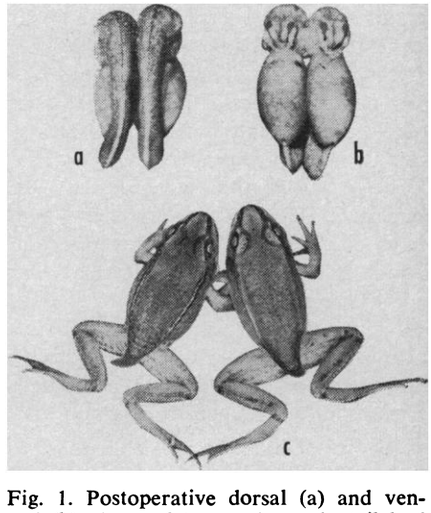

Fig. 1. Postoperative dorsal (a) and ventral (b) views of two embryos in tail bud development (68 hours after fertilization at 20°C) joined in parabiosis in the region of the gill primordium. The connection between the parabionts becomes severed during the closing stages of metamorphosis (c). (In this particular case, the paired members are nonspotted or burnsi frogs, well-known pattern mutants of the common-spotted leopard frog.)