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

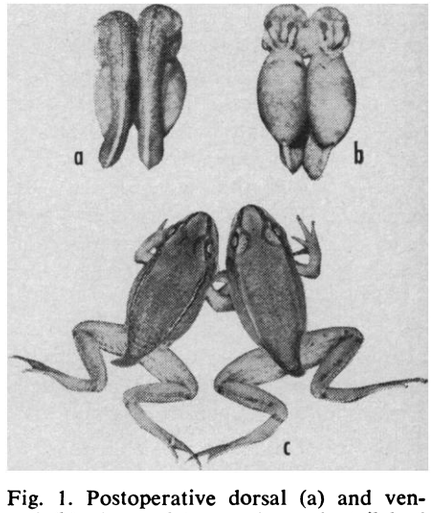

Fig. 1. Postoperative dorsal (a) and ventral (b) views of two embryos in tail bud development (68 hours after fertilization at 20°C) joined in parabiosis in the region of the gill primordium. The connection between the parabionts becomes severed during the closing stages of metamorphosis (c). (In this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

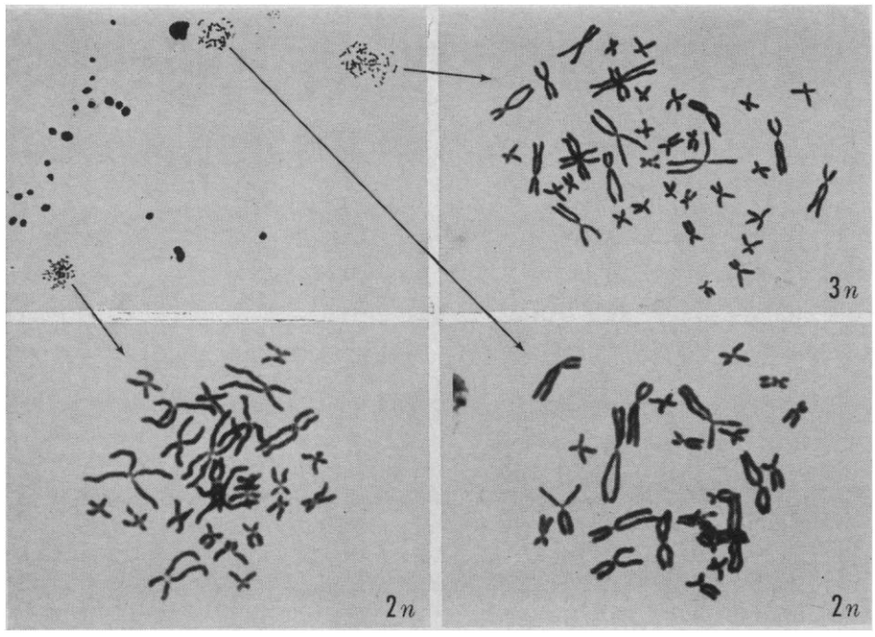

Fig. 2. Low-power view (upper left) of an air-dried, Giemsa-stained preparation of cultured leucocytes from peripheral blood of an ex-parabiotic frog, accompanied by enlarged views of the three metaphase plates in the microscopic field to reveal the chimeric composition of the leucocyte population. ($2n = 26$ chromosomes; $3n = 39$.)

(*8*). A triploid embryo was united side-to-side with a diploid embryo in the region of the gill primordium to ensure vascular communication (Fig. 1, a and b). Seventeen parabiotic pairs were successfully carried through metamorphosis. During their transformation into young frogs, the co-parabionts became disjoined (Fig. 1c). Skin grafts exchanged between the separated members of a pair have been fully tolerated. The state of tolerance is characterized by a high degree of individual specificity. That is to say, the ex-parabiotic frogs have accepted skin grafts only from their former partners.

The ex-parabionts were tested for chimerism 1 month after they had separated from each other, and again 6 months after their separation. On each occasion, blood was drawn from the femoral artery of the thigh of each ex-parabiont, and the leucocytes were cultured for analysis of their chromosomes. The culture medium that has proved most satisfactory is one slightly modified after Jaylet (*9*). It has the following composition: 100 ml of Eagle's minimum essential medium (Grand Island Biological Co.), 5 ml of phytohemagglutinin M (Difco) rehydrated with hemagglutination buffer, 6 ml of whole egg ultrafiltrate (Microbiological Associates, Inc.), 5 ml of penicillin-streptomycin mixture (5000 units/ml), 11.8 ml of calf serum (Grand Island Biological Co.), and 30 ml of a 2 percent water solution of lactalbumin hydrolyzate (Difco). Three to four drops of whole blood were incubated in 5 ml of the culture medium for a period of 72 hours at 26°C. Six to eight hours before the cells were harvested, 0.25 ml of colchicine solution (0.1 g/100 ml of water) was added to each culture. Two techniques for chromosome preparations were used with equal success: the hypotonic citrate–air drying technique of Moorehead *et al.* (*10*) and a modification of this technique described by Fox and Zeiss (*11*). The concentration of sodium citrate prescribed in the procedure of Fox and Zeiss was increased to 1.12 percent, as suggested by Seto *et al.* (*12*) for optimum pretreatment of amphibian leucocytes.

The results were unfailingly consistent. The chromosome preparations of the cultured leucocytes of each ex-parabiont contained both diploid ($2n = 26$) and triploid ($3n = 39$) metaphase plates. Figure 2 shows a microscopic field with three metaphases, two of which are diploid and the other triploid. More than 100 figures were scored in each preparation to obtain a crude measure of the relative proportions of diploid and triploid cells. There is doubtless an element of bias in the selection of figures, and the cells in mitosis may not be representative of the population as a whole. Nevertheless, it can be said that the "foreign," or donor-type, leucocytes constitute at least 15 percent of the blood cell population of each host. Moreover, as revealed by retests, the frequencies of the donor-type leucocytes do not change appreciably with age, at least up to 6 months after metamorphosis.

The presence of circulating leucocytes of two genetically distinct kinds in ex-parabionts that have been apart for as long as 6 months is strong presumptive evidence that the donor-type leucocytes are descendants of primordial blood cells that had been conveyed through vascular anastomoses into the circulation of the host in embryonic life and had settled in the host's hematopoietic tissues. Each ex-parabiont thus has blood-forming tissue capable of producing two kinds of blood cells, its own kind and that of its former partner. This view is necessarily tempered by the absence of information on the life span of any member of the leucocyte series in the leopard frog. Accordingly, the possibility cannot be discounted that certain types of leucocytes have exceedingly long lives as circulating cells and have persisted in the host from the time of their initial introduction without ever having homed in the host's hematopoietic tissues. We may conclude by saying that our interpretation of the "seeding" of hematopoietic stem cells is eminently reasonable but cannot be considered as definitive (see *13*).

The deliberations above should not obscure the salient findings of the study: ex-parabiotic frogs have mixtures of two genetically distinct leucocytes. Only skin homografts having the same genetic origin as the donor-type leucocytes are tolerated. It seems likely that blood cell chimerism is a requisite for the mutual specific tolerance to skin homografts exhibited by ex-parabiotic pairs of frogs.

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13. It is highly unlikely that leucocytes are the only cells that are interchanged between the co-parabionts. We may reasonably surmise that the ex-parabiotic frogs are also erythrocyte chimeras. The frog's erythrocytes are nucleated, but they have not been observed to divide in vitro. Active mitoses of circulating red cells in vivo have been witnessed [D. C. Braungart, *J. Hered.* **41**, 203 (1950)], but such occurrences must be rare.
14. Supported by grant GM-11782 from the PHS and by grant IN-24-I from the American Cancer Society.

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Simple Photoreceptors in Limulus polyphemus

Abstract. *The "olfactory nerve," the endoparietal eye, and the rudimentary lateral eyes of* Limulus (polyphemus) *contain simple photoreceptor cells that duplicate many of the electrical responses of the retinular cells of the lateral eye; the responses are a receptor potential consisting of an initial transient phase and a subsequent steady phase, low-amplitude fluctuations, and a small locally regenerative response to pulses of both light and current. Photic stimulation does not induce conducted action potentials, but does increase the membrane conductance. The receptor potential requires the presence of sodium ions in the external medium. Measurements of action and absorption spectra indicate a photopigment whose maximum absorption is of light with wavelength of 535 nanometers. The functional significance of these cells has not been ascertained.*

Gwilliam and Fahrenbach (*1*) have demonstrated that the eyes of barnacles are simple photoreceptors that are not organized into ommatidia. We now describe three analogous structures, the so-called olfactory nerves, the rudimentary lateral eyes, and the endoparietal eye, in *Limulus polyphemus*, and summarize our initial studies on their physiology.

The "olfactory nerves" in *Limulus* (*2*) consist of three bundles—two lateral and one medial—which run forward and ventrally from the protocerebrum to an integumentary structure of unknown function, variously called a ventral eye or olfactory organ (*3*). Large cell bodies (100 to 300 μ) are scattered along the desheathed lateral nerves. Our electron-microscopic studies show the presence of microvilli on the margins of the cell bodies.

The rudimentary lateral and endoparietal "eyes" are structurally very similar to each other (*3*). There are two of the former, one behind and internal to each compound lateral eye, and one of the latter, just behind and beneath the two external median eyes. These organs are easily recognized by the glistening white masses of connective tissue in which the photoreceptor cells are enmeshed. In each case, a tough sheath surrounds the whole organ. The axons are large and unbranched, those of each rudimentary eye running in two bundles into the nerve of the lateral eye and those of the endoparietal eye forming two bundles in the nerve of the median eye.

Impalement of these cells with microelectrodes is difficult unless the tough sheaths are removed or softened with trypsin. The former method is used on the olfactory nerves; the latter is used on the rudimentary and endoparietal eyes. Resting potentials in cells which give stable responses vary from −25 to −53 mv. A typical receptor potential is seen in Fig. 1*a*. There are two components: (i) an initial, transient depolarization which sometimes overshoots zero by as much as 15 mv; and (ii) a relatively flat steady phase which is always negative. Both the time-course and the amplitude of these components are affected by the degree of dark adaptation. For a light stimulus of constant intensity, the amplitude of the steady phase rapidly increases during the first 15 seconds of adaptation to dark and subsequently becomes time invariant. The amplitude of the transient phase rapidly increases for the first 60 seconds and continues to increase, although at a lower rate, for as much as 15 minutes. Cells fully adapted to the dark have a long transient phase (about 500 msec) while light-adapted ones show a sharper, faster transient phase (200 to 300 msec). Cells adapted to light usually exhibit a small repolarization between the end of the transient phase and the beginning of the steady phase.

Low-amplitude fluctuations, similar to those seen in the lateral eye of *Limulus* (*4*), appear when we allow the cells to adapt to the dark for 5 to 10 minutes (Fig. 1*b*). The amplitude of the fluctuation ranges from 1 mv to as high as 20 mv. Flashes of low intensity (large enough to give a receptor potential) reduce the fluctuations to zero.

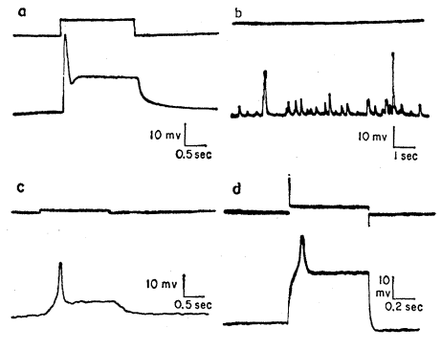

Fig. 1. Intracellular records from olfactory nerve cells. Upper trace monitors stimulus; lower trace monitors response. *a*, Receptor potential from light-adapted cell; *b*, low-amplitude fluctuations in dark-adapted cell; *c*, small spike-like response on steady phase of receptor potential with low-intensity light pulse; and *d*, spike-like response to depolarizing current pulse (approximately 10^{-8} amp). Experiments conducted at 20°C. The transient component of the receptor potential (*a*) should not be confused with the spike-like responses (*c* and *d*). Zero voltage for the lower trace (the response) is the base line of the upper trace (the stimulus monitor).

In cells adapted to the dark for about 60 seconds, the amplitude of the steady phase is a linear function of the logarithm of the light intensity throughout the range tested (Fig. 2). However, the amplitude of the transient phase is logarithmic only over the lower portion of the range and seems to become "saturated" in the upper portion.

The spectral sensitivity of the olfactory nerve cells, as determined by intracellular electrodes, is given in Fig. 3

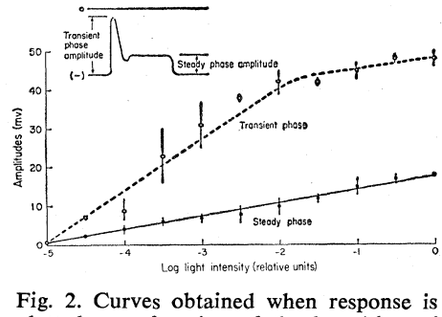

Fig. 2. Curves obtained when response is plotted as a function of the logarithm of the intensity for transient- and steady-phase components of receptor potential from olfactory nerve cells.