despite rather extensive studies have seen none in bone matrix or in relation to osteocytes. I have not found nerve endings.

The very magnitude of bone innervation implies important functions of nerves in bone dynamics.

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18 January 1968

Germ Cell Chimerism: Absence in Parabiotic Frogs

Abstract. *Embryos of the leopard frog deprived of primordial germ cells by treatment with ultraviolet light were joined in parabiosis with normal, unirradiated embryos. The irradiated member of the pair was not colonized by germ cells from its normal partner. Unlike the primordial germ cells of birds and mammals, the germ cells of frog embryos are not carried by the circulating blood.*

The last decade has witnessed a resurgence of interest in the migratory behavior of primordial germ cells in vertebrate embryos. Simon (*1*), Meyer (*2*), and Singh and Meyer (*3*) have demonstrated convincingly that the primordial germ cells of birds are conveyed in the bloodstream from their site of origin in the extraembryonic germinal crescent to the gonadal primordium within the embryo proper. The germ cells of mammals have been claimed (*4*) to reach their final destination by active, amoeboid-like movements through the embryonic tissues. Until recently there was scarcely any hint that mammalian germ cells enter the vascular channels. In 1962, Ohno and his colleagues (*5*) were surprised to find female or XX-bearing gonial cells in the testes of newborn dizygotic twin calves known to be chimeric for blood cells. Apparently, the anastomosis of placental vessels during the embryonic life of the cattle twins permits not only the interchange of primordial blood cells but also the cross circulation of primordial germ cells. Subsequently, Ohno and Gropp (*6*) furnished histochemical evidence of blood-borne primordial cells in the cattle embryos, and Benirschke and Brownhill (*7*) uncovered germ cell chimerism in another mammal that regularly produces twins, the marmoset.

It has long been assumed that the yolk-rich germ cells of amphibians do not have sufficient amoeboid activity to migrate singly through other tissues (or to invade blood vessels) and that these cells are simply displaced passively to their final position in the genital ridges (*8*). Yet the separation of primordial germ cells (of *Rana pipiens*) from the future intestinal cells occurs in the vicinity of major blood vessels (*9*). At or about embryonic stage 20 (*10*), the primordial germ cells, recently isolated from the gut (endoderm) wall, lie directly beneath the dorsal aorta and medial to the large right and left cardinal veins. There has been no experimental evidence to confirm or deny the possibility that the germ cells can or do enter, or even occasionally wander, into the blood channels.

In 1966, Volpe and Gebhardt (*11*) demonstrated that leopard frog embryos joined in parabiotic union were chimeric in later life with respect to their blood cells. In the same year, Smith (*12*) perfected a technique for producing frog embryos that are devoid of primordial germ cells by exposing the vegetal hemispheres of fertilized eggs to ultraviolet light at the time of the first cleavage division. Irradiation with ultraviolet light destroys the germ cell determinants localized in the vegetal cortical region of the fertilized egg (*13*). Thus, possible transport of primordial germ cells in the common circulation between parabionts could be explored by joining irradiated embryos with normal embryos and determining whether the gonads of the irradiated larvae are repopulated with primordial germ cells from the normal partners. Our study was greatly facilitated by Smith's observation (*12*) that primordial germ cells can be unequivocally identified in the living animal at embryonic stage 25 (Fig. 1).

We freely adapted Smith's procedure for obtaining embryos free of germ cells. Ovulation was induced in adult leopard frogs (*Rana pipiens*) by injections of whole pituitary glands (*14*), and eggs were extruded into a sperm suspension prepared by macerating a pair of testes in 10 percent Ringer solution. In each experimental trial, the jelly membranes were removed with watchmaker's forceps from approximately 100 eggs immediately after fertilization. The denuded eggs were transferred in a wide-mouthed pipette to a quartz microscope slide (3 by 1 inches, Thermal American Fused Quartz Company, Montville, N.J.), at each end of which was placed a thin roll of clay (permoplast). The eggs were permitted to rotate so that the vegetal hemispheres were downward, and excess Ringer solution was withdrawn from

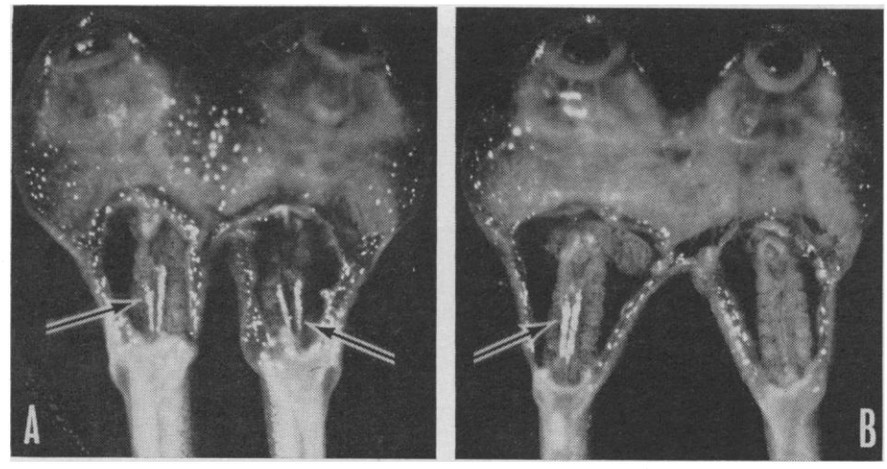

Fig. 1. (A) Unirradiated parabionts at Shumway stage 25; both embryos possess two conspicuous rows of whitish, large, primordial germ cells (indicated by arrows). (B) Irradiated embryo (right), devoid of primordial germ cells, joined to an unirradiated embryo (left) having two prominent rows of germ cells.

the slide with a Pasteur pipette. A second quartz slide was placed on top of the first, and the eggs were flattened between the two slides by exerting firm but gentle pressure on the ends of the upper slide over the permoplast.

The slides were placed at a distance of 18 inches (1 inch = 2.54 cm) directly over an ultraviolet lamp (Mineralite model R-51). This particular model delivers 155 μw/cm^2 (or 930 erg min^{-1} mm^{-2}) at a distance of 18 inches. Germ cell formation was completely suppressed when the vegetal hemispheres were exposed to the irradiation for 45 minutes. Parabiosis in the future gill region was accomplished at the tailbud stage of development (stage 17 of Shumway, *10*) by joining unirradiated embryos with either irradiated (experimental) embryos or other unirradiated (control) embryos. At Shumway stage 25, the parabionts were anesthetized in MS-222 (1:10,000 concentration of tricaine methanesulfonate; Sandoz Pharmaceuticals, Hanover, N.J.), carefully eviscerated, and photographed.

The results of eight replicate experiments were uniformly consistent. Unirradiated pairs of embryos examined at Shumway stage 25 invariably displayed normal gonad development. Each embryo possessed two conspicuous genital ridges containing large primordial germ cells (Fig. 1A). In marked contrast to unirradiated pairs of embryos, no primordial germ cells were seen in irradiated embryos joined to normal partners possessing the usual two rows of primordial germ cells (Fig. 1B). Only sterile genital ridges were present in the irradiated embryos.

The observed absence of primordial germ cells in irradiated parabionts is interpreted as indicating that the circulatory system does not transport germ cells in the embryos of *Rana pipiens*. The possibility exists that a few germ cells might gain entry into the circulating blood but remain undetected because of their failure to reach the definitive site in the genital ridges. This seems unlikely, however, particularly if the successful colonization of primordial blood cells in hematopoietic organs in parabiotic frogs is taken as a dramatized demonstration of the strong homing tendency of cells (*15*).

Negative data are rarely satisfying, but we may conclude by stating that our results do not lend support to a thesis that would advocate the migration of amphibian germ cells through blood vessels. The explanation that now seems most reasonable is Humphrey's contention, dating back to 1925 (*8*), that the primordial germ cells of amphibians reach their final position in the genital ridge entirely by mechanical displacements accompanying embryonic growth.

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29 January 1968

Chick's Response to an Imprinting Stimulus: Heterosis and Evolution

Abstract. *Tendencies of day-old chicks to respond toward, to approach, and to stay near a distant audiovisual imprinting apparatus were shown to be inherited in a heterotic manner. High correlations found among the traits suggest that these behavioral responses are not independent but sequential. The fitness value of such a behavioral sequence is explained in terms of imprinting.*

The formation of initial social bonds by precocial neonates is a form of behavior imprinting which, although widely investigated, remains controversial; the phenomenon consists of a complex of events. Thus investigators must either go to great lengths to show that the trait quantified is really related to imprinting, or (more often) speak of such factors as the "approach response" and "the following response," which are assumed to be related to imprinting. Although approach and following may be involved, maintenance of the following response may not necessarily reflect a preference for the object followed (*1*), and response and approach to an imprinting apparatus have not been shown to be a measure of a chick's tendency to imprint (*2*).

Our purpose was to determine whether response toward, approach to, and tendency to stay near a parent surrogate during the critical period for imprinting are adaptive traits, and to determine the relations among these traits. Demonstration of heterotic inheritance of the traits measured would greatly strengthen the argument that they are components of imprinting.

The subjects were 669 domestic chicks from five 2 × 2 mating sets (*3*). Since one of the lines was used in two mating sets, there were nine types of F_1 purebreds (290 chicks) and ten types of F_1 crossbreds (379 chicks). Eggs were collected daily and stored at 13°C until incubation; after 18-day incubation they were placed in individual compartments in a dimly lighted hatcher (maximum light intensity, 4.3 lux). Compartments were inspected at 2-hour intervals, and when a chick had hatched (completely emerged from the egg) it was transferred to a labeled compartment until testing.

The testing compartment, developed from one described (*4*), was hexagonal and measured 90 cm between parallel sides. The visual stimulus consisted of a translucent plastic dome containing moving colored lights that flickered on

Table 1. Weighted phenotypic correlations among components of the approach response; *N*, 535.

	Response, no response	Time to approach	Approach, no approach	Time near apparatus
Time to respond	.81	.75	.80	—.81
Response, no response		.67	.70	—.54
Time to approach			.96	—.83
Approach, no approach				—.78