aggregated on the sieve plates in variable amounts; some emerges in the exudate. This finding may explain the high nitrogen content of phloem exudate of squash (*13*), the fact that the exudate coagulates, and the protein reaction of slime in microchemical tests. Broken-down membrane material may account for it being termed "lipoprotein" or "steroid."

Perhaps the old problem of strands in sieve tubes has finally been resolved (*12*, *14*). Since the individual filaments of the permanent network are far too small for resolution by the light microscope, the impression that strands existed in sieve tubes is understandable; under many conditions one could not see or photograph the strands. The coarser strands seen in some photographs probably represent aggregations of filaments into large fibrils of the type shown (*7*, fig. 17). Occasionally these strands can be seen stretched between sieve plates and extending through sieve pores (*12*, *14*, *15*). The distinction between slime and this filamentous reticulum is becoming increasingly apparent in many electron-microsope studies of phloem.

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15 January 1968

Nerves in Cortical Bone

Abstract. *Modified electron-microscopic techniques permit the sectioning of fully mineralized compact bone. Cortical canals in human femur are extensively innervated. Most nerves are unmyelinated and range from 0.5 to 10 micrometers in diameter. I have found a few mixed nerves (myelinated and unmyelinated fibers), one of which was 130 micrometers in diameter.*

Despite its important implications, we know little about bone innervation. The technical difficulties with and limited scope of "special nerve stains" contribute to this lack of understanding.

Using modified electron-microscopic techniques, I have sectioned fully mineralized compact bone for ultrastructural studies (*1*). Nearly all haversian canals of adult dogs contain unmyelinated nerves 0.8 to 7 μm in diameter. The diameters of individual nerve fibers range from 0.25 to 0.6 μm. I have now examined fully mineralized cortex of the adult human femur. It is extensively innervated.

The cortical canals contain unmyelinated nerves 0.5 to 10 μm in diameter. They consist of fibers (axons) invaginated into recesses in the Schwann cell plasma membrane. The fibers contain mitochondria, 400- to 800-Å vesicles, and neurofilaments about 115 Å in diameter. A basement membrane 400 Å thick surrounds the nerve, and endoneurial collagen fibrils surround the basement membrane.

One canal contained a mixed nerve (myelinated and unmyelinated fibers) 130 μm in diameter (Fig. 1). A few other canals contained one myelinated fiber adjacent to one or more capillaries. The myelinated fibers are 5 to 9 μm in diameter.

Numerous investigators have demonstrated nerves in bone by gross dissection (*2*), routine histologic sections (*3*), and methylene blue and silver stains (*4*). Others suspected their presence as a result of clinical observations, especially during bone surgery with local anesthesia (*5*). Various investigators have disagreed about the exact distribution of these nerves. Most of them demonstrated periosteal nerves, nerves in trabecular bone, and fibers entering cortical canals, but technical limitations prevented them from determining much about the extent of the nerve supply in the haversian canal. Some thought nerves extended into bone matrix, and others thought fibers were closely related to bone cells. I have only seen the nerves in haversian canals and

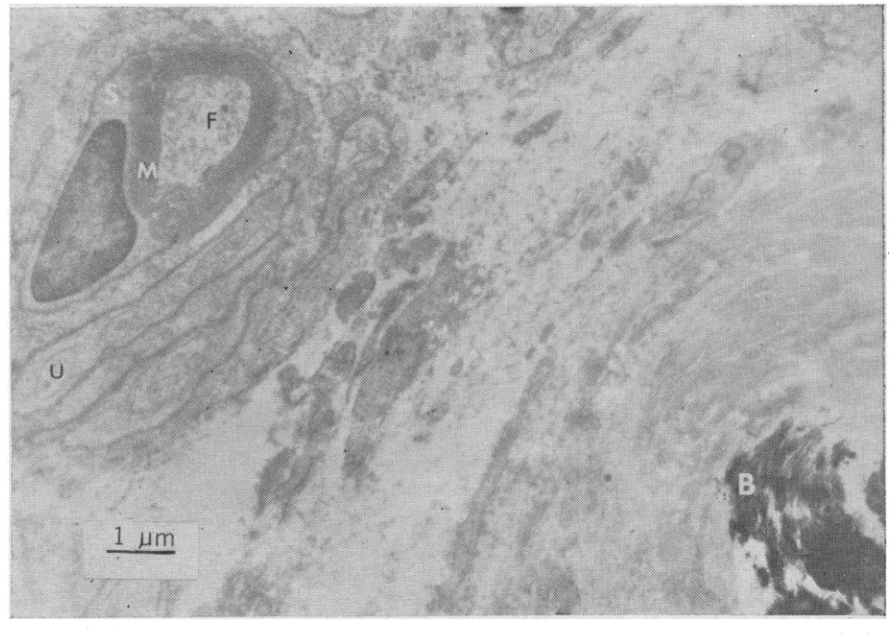

Fig. 1. Portion of a cortical canal containing a mixed nerve. Surgical biopsy of the distal femur of a 54-year-old woman. Note the mineralized bone matrix (*B*) which forms the canal wall. Unmyelinated fibers (*U*) are invaginated into recesses in the Schwann cell plasma membrane. The myelinated fiber consists of a central nerve fiber (*F*) containing neurofilaments surrounded by layers of myelin (*M*). This is surrounded by the Schwann cell (*S*) and a basement membrane.

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

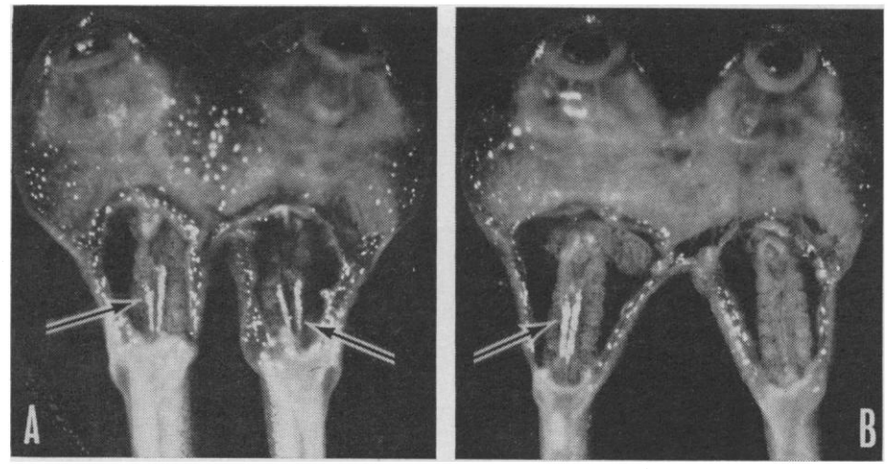

Fig. 1. (A) Unirradiated parabionts at Shumway stage 25; both embryos possess two conspicuous rows of whitish, large, primordial germ cells (indicated by arrows). (B) Irradiated embryo (right), devoid of primordial germ cells, joined to an unirradiated embryo (left) having two prominent rows of germ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