are necessary to establish the cellular and molecular basis of the defect in antibody production in copper-deficient mice.

The effect of copper deficiency on the immune response appears to be distinct from the effects of deficiencies of other trace metals (zinc and iron) known to affect immune function. Zinc deficiency causes a decrease in the number of antibody-producing cells which appears to be dependent on T cell helper function (*9*). In zinc-deficient mice, the spleen and thymus are atrophic. Our copper-deficient mice, with impaired immune function, had normal or enlarged spleens (Table 1) and normal-appearing thymus glands. Furthermore, zinc metabolism is unaltered during copper deficiency (*10*). Iron deficiency appears to alter humoral-mediated immunity (*11*); thus the defects in iron metabolism in our copper-deficient mice may explain the impairment in antibody production. This is unlikely, however, since some $-Cu_2$ mice that had only mild anemia and normal liver iron levels still had impaired immune response (Table 1 and Fig. 1). Multiple deficiencies in copper, iron, and zinc may occur in humans and result in impaired immunity due to a combination of mechanisms.

The many physiological roles of copper are evidenced by the numerous functional and structural alterations caused by its deficiency. Extensive investigations into the biochemical mechanisms have led some to theorize that reductions in copper-dependent enzymes such as ceruloplasmin, cytochrome oxidase, superoxide dismutase, lysyl oxidase, tyrosinase, and dopamine β-hydroxylase are responsible for the pathological expressions of copper deficiency (*12*). We do not know which, if any, of these enzymes might be involved in the immune response. However, it is clear that copper is another nutritional factor necessary for a competent immune system.

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8 April 1981

Role of Golgi Apparatus in Sorogenesis by the Cellular Slime Mold *Fonticula alba*

Abstract. *Aggregating cells of the cellular slime mold* Fonticula alba *form a volcano-shaped fruiting structure which at maturity bears its spores apically in a globose mucous mass. Numerous dictyosomes forming in the sorogenic cells are involved in the accumulation and deposition of stalk material.*

In *Fonticula alba*, a recently described cellular slime mold (*1*), the trophic cells bear certain resemblances to the amoebas of both acrasid and dictyostelid cellular slime molds, but the form and organization of the sorocarp is unique. Major differences between acrasids and dictyostelids have been described elsewhere (*2*). The amoebas of *F. alba*, like those of the dictyostelids, form filose pseudopodia when placed under moist conditions. However, each cell, unlike those of the dictyostelids, has a nucleus with an inconspicuous nucleolus and mitochondria with discoid cristae similar to those of certain acrasids. The fruiting body with its volcano-shaped stalk is unlike any found among other cellular slime molds, with the possible exception of the acrasid *Guttulinopsis nivea* (*3*).

The fruiting stage begins when trophic amoebas cease feeding and form dense aggregates within or slightly on top of masses of amoebas and bacteria present on the agar surface. Once the cells have aggregated, they envelop themselves in a large mass of mucus-like material (Fig. 1A). A group of amoebas near the top of the aggregate rises upward as the developing stalk stretches into a slender projection (Fig. 1B). The base of the sorocarp is very thick and contains a large number of cells (Fig. 1E). As the amoebas move upward they synthesize and eject stalk material. While the cells are embedded in a portion of this material, the majority of it accumulates in the walls of the sorocarp, much of it within the basal area. The movement of cells leading to formation of the stalk neck continues until the neck achieves a height roughly proportional to the diameter of the base and then ceases. At this point, many of the cells inside the stalk cease making stalk material, become round, and develop spore walls around themselves. A small opening appears at the top of the stalk and the spores flow out into a large mucous mass atop the stalk (Fig. 1, C and D).

In order to fully understand the process of sorogenesis in *F. alba*, it is necessary to determine how the stalk is formed. Representative examples of the stages of sorogenesis were selected for observation by electron microscopy. Small plugs of the agar on which individual sorocarps were developing were covered with molten 2 percent pure agar. When the agar had solidified, completely immobilizing the sorocarps, the plugs were fixed in 2 percent glutaraldehyde buffered in 0.1*M* sodium cacodylate for 30 minutes at room temperature and then for 90 minutes at 4°C. The specimens were rinsed twice in 0.1*M* sodium caco-

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dylate and then postfixed in cold osmium tetroxide in the buffer for 2 hours. They were rinsed twice in buffer, dehydrated in ethanol followed by acetone, and finally embedded in Spurr epoxy resin. Sorocarps in various stages of development were sectioned longitudinally for electron microscopy.

Ultrastructural analysis of sorogenic amoebas reveals that very early in sorogenesis these cells are stimulated to form numerous dictyosomes from the outer membrane of the nuclear envelope and that these organelles appear to be very active in the accumulation and deposition of stalk material. Numerous dictyosomes form in almost every cell early in sorogenesis (Fig. 1F), even before the transition to the sorogenic phase is complete. Small rounded vesicles move from the outer membrane of the nuclear envelope toward each dictyosome (Fig. 1G). The presence of bacteria within and outside these cells and the fact that the sorocarps arise from thick mounds of bacteria indicate that starvation is not a motivating factor in sorogenesis in this species, as it is in the dictyostelids.

The Golgi apparatus of *Fonticula* shows a pattern of development that is

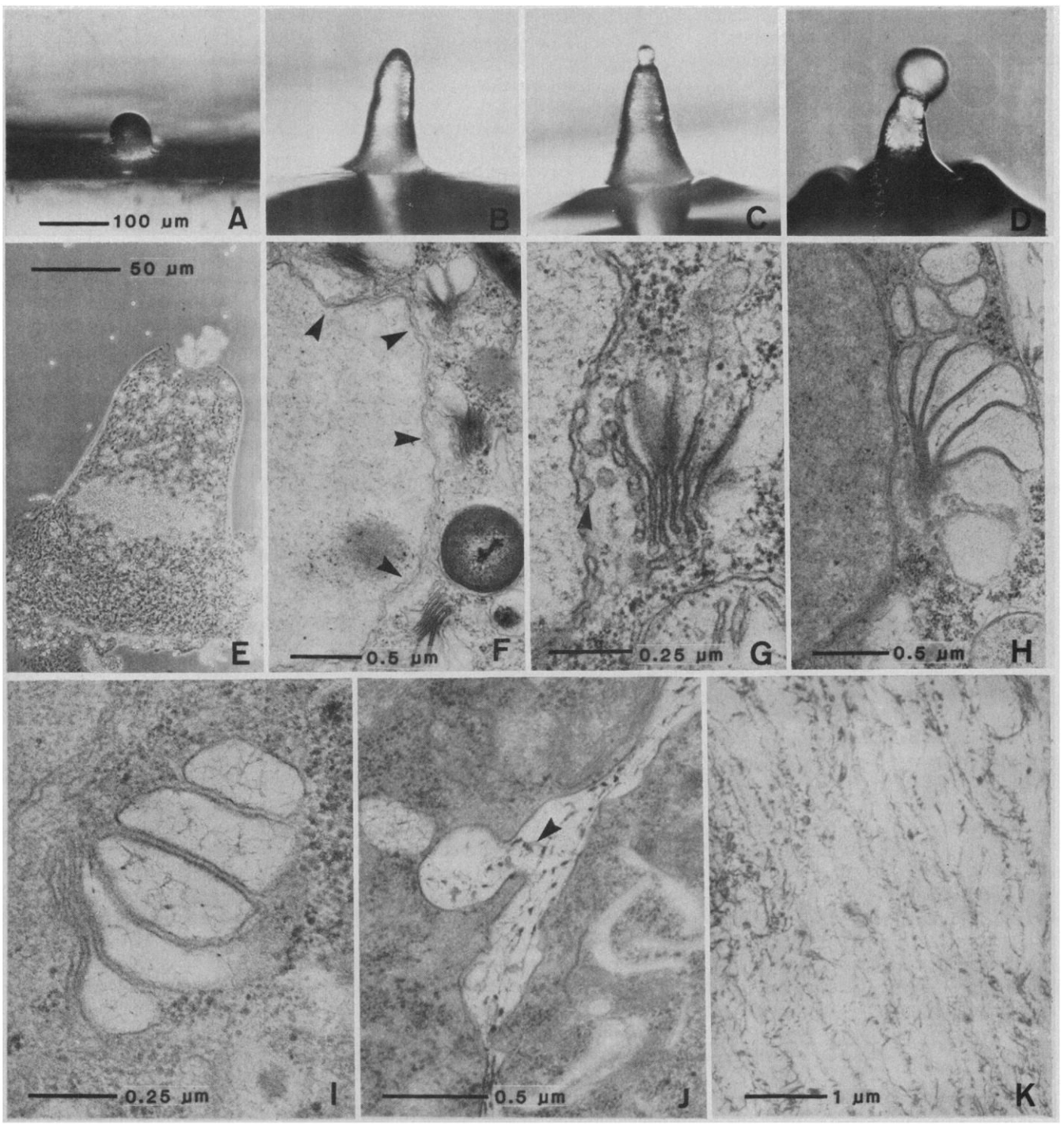

Fig. 1. Sorogenesis in *Fonticula alba*. (A) Aggregating amoebas form a mass of mucous material around themselves. (B) Amoebas near the top of the aggregate rise upward and form the stalk neck. (C) At culmination, an opening occurs at the stalk apex and the spores flow out. (D) Spores in a mucous matrix on top of the stalk. (E) Stalk that has been removed from the agar surface. Note the thickened base. (F and G) Dictyosomes (arrows) forming from outer nuclear membrane of a sorogenic cell. (H and I) Thread-like material develops within the dictyosome vesicles. (J) A vesicle empties its material into the area (arrow) between two cells. (K) Wall of stalk neck with abundant fibrillar material.

unique among the cellular slime molds. Instead of small vesicles pinching off from the ends of the individual cisternae, the latter swell up completely so that they resemble the sections of a citrus fruit (Fig. 1H). All the cells within the young sorocarp have many of these stacks of swollen vesicles. A thread-like material embedded in a mucous matrix develops abundantly within the vesicles (Fig. 1I). Substances very similar to that contained within the vesicles can be seen between the individual cells and in the walls of the stalk surrounding them (Fig. 1, J and K). The vesicles appear to migrate to the plasma membrane of the cell, fuse with it, and release their contents to the outside (Fig. 1J). While much of the mucous material containing some thread-like matter forms the matrix around the cells, most of the thread-like material accumulates in the walls of the neck (Fig. 1K) and especially in the thickened base of the stalk. This is believed to give support to the stalk and its sorus.

While the sorocarp is developing and the cells are moving upward during stalk formation, all the cells have a fairly amoeboid appearance. However, after the sorocarp has achieved its full height, the cells within begin to show a division of labor. Those cells nearest the base are amoeboid and continue to produce dictyosomes that accumulate and deposit stalk material. Those farther up are much more rounded, have very few dictyosomes, and are beginning to develop into spores. The cells in the upper neck are elliptical, lack dictyosomes, and are forming thick walls as they mature into spores.

At the culmination of sorogenesis, the encysted cells emerge from the apex of the stalk into a mucous matrix and become the sorus. At this point, the neck region of the sorocarp is almost devoid of cells. However, a group of amoeboid cells remains in the base and continues to manufacture stalk material even after the spores emerge. It seems likely that the mucous material surrounding these cells may absorb moisture from outside the sorocarp and thereby exert enough pressure within the stalk to cause it to elongate and the spores to rise and be expelled. Evidence of this was obtained by removing immature sorocarps from the bacterial culture and placing their bases in contact with a layer of water on a slide. The developing spores and mucous material rapidly emerged from the stalk apex. A similar mode of cell movement, in which the sorogenic cells eject hydrophilic material that swells and causes the cells to rise in the formation of an aerial fruiting body, was recently reported in an unrelated organism, the ciliate *Sorogena stoianovitchae* (*4*).

The origination of dictyosomes from the outer membrane of the nuclear envelope is an uncommon but not unique phenomenon. Although the Golgi apparatus originates from the endoplasmic reticulum in most organisms investigated, its formation from the nuclear envelope has been demonstrated in several systems, including sorogenesis in apparently unrelated cellular slime molds (*5*, *6*) and the embryonic stages of some higher plants and animals (*7*). The aggregating amoebas of the acrasids *Guttulinopsis nivea* (*5*) and *Copromyxa protea* (*C. arborescens*) (*6*) form structures from the nuclear envelope resembling rudimentary dictyosomes. Also, in *Endemosarca hypsalyxis*, an organism possibly related to plasmodiophorids, small vesicles termed "amplexi" migrate from the nuclear envelope to form small dictyosome-like structures (*8*). However, in each case these dictyosomes remain very small and do not bear any resemblances to the large vesicles seen in sorogenic cells of *F. alba*.

Perhaps the more significant observation is that the sorogenic cells of *F. alba* are stimulated to form conspicuous, unusually shaped dictyosomes at the beginning of aggregation, that these organelles function in the accumulation of stalk material, and that they disappear before the cells encyst. This series of events occurs only during one specific phase of the life cycle. The stimulus that elicits such an intriguing response remains to be identified.

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24 February 1981; revised 6 April 1981

Mesenchymal Cells from the Human Embryonic Palate Are Highly Responsive to Epidermal Growth Factor

Abstract. *An established line of mesenchymal cells from the human embryonic palate is highly sensitive to the stimulatory effect of epidermal growth factor on growth, labeled thymidine incorporation, and ornithine decarboxylase activity. The results suggest that epidermal growth factor may play a key role in development of various human embryonic and fetal tissues.*

Formation of the secondary palate occurs early in human development (weeks 6 to 8 of gestation) and involves a complex series of steps involving both the growth and differentiation of epithelial and mesenchymal cells (*1*). These events are presumably under the control of a number of hormones and growth factors (*2–4*). In the study described here we investigated the effects of epidermal growth factor (EGF) on growth and other parameters of an established line of human embryonic palatal mesenchymal (HEPM) cells cultured in a serum-free hormone-supplemented medium and on the binding of EGF to cell surface receptors.

The HEPM cell line (CRL 1486) was established for us by the American Type Culture Collection (Rockville, Maryland). These mesenchymal cells, pre-

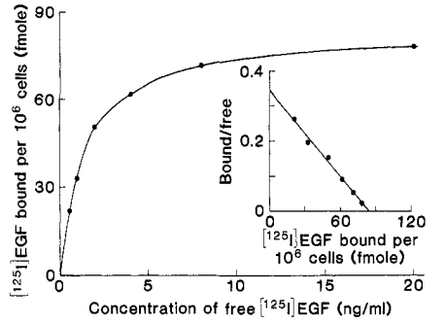

Fig. 1. Specific [^{125}I]EGF binding to HEPM cells. The HEPM cells were plated at a density of 2×10^5 cells in 3 ml of complete DMEM in 35-mm dishes and grown for 72 hours as described. Labeled EGF binding experiments were carried out according to the methods reported previously (*24*).

0036-8075/81/0731-0563$00.50/0