Dear Editor:

The Phylum Porifera (sponges) represents the simplest and the most primitive living Metazoa. Sponges are considered to be a link between unicellular and pluricellular organisms, and as such they represent an interesting point in evolution of cell integration and of interactions among cells organized in higher functional structures. Sponges do not have fully defined tissue or organs and all their functions are based only on cellular systems.

The classical studies done by Wilson (13) on reaggregation of sponge cell suspensions, and subsequent regeneration of functional sponges, have opened extensive research on the specificity of sponge cell recognition and adhesion (7), on the sorting of cells in heterogeneous cell aggregates (6), and on determination or differentiation stability of cells isolated from larvae or from adult organisms (2,4). The aim of all these studies was to obtain functional sponges from isolated cells.

Recent research on sponges have renewed interest in development of permanent sponge cell cultures. It has been shown that the sponge cells recognition and adhesion are controlled by elaborate molecular systems analogous to calcium-mediated adhesion in vertebrate cells (7,9). These systems are integrated with intracellular and intercellular messenger pathways (9), the evolution of which is of considerable interest in understanding communication among cells in multicellular organisms. On the other hand, biochemical studies have shown that sponges can use alternative metabolic pathways (1,8), with synthesis of molecules not identified in other organisms (2,4). The aim of all these studies was to obtain functional sponges from isolated cells.

Cell suspensions of Polymastia were composed mainly of rounded Choanocytes, many of which bear still their active flagellum, even after cooling and transport to the laboratory. Some cells formed small aggregates; the subsequent organization, differentiation and evolution of cell aggregates into functional structures was never observed. Individual ameboid cells were recognized in suspension, forming blunt, linear and rather rigid pseudopodia (Fig. 1 C). After two days, the ameboid cells were present at the culture flask bottom (Fig. 1 D). They moved actively on the substrate, forming a typical lamellipodial fringe at the anterior pole, and short rounded pseudopodia at the posterior pole. They displayed a high phagocytic activity, and digestive vacuoles were prominent in their cytoplasm. They detached frequently from the culture flask bottom, floating in the medium, with formation of thin filopodia, and adhered subsequently to the substrate. Their diameter was approximately 60 µm.

With the regular changes of medium containing non-adherent and dead cells, ameboid cells adherent to the bottom were the only cell type observed. They divided actively, either floating in medium, or on the flask bottom, separating by an active movement in opposite directions, until interruption of the remaining thin cytoplasmic link (Fig. 1 E). The maximal cell density reached in cultures was close to 5 × 10^5 cells/25 cm² tissue culture flask; after reaching it, cells entered in a stationary phase of growth. This density is lower than that observed for proliferation-inhibition of vertebrate cells, and is not mediated by cell-cell contact.

Cell suspensions obtained from Polymastia were composed of small rounded cells, among which choanocytes could not be easily recognized. Ameboid cells appeared on the culture bottom after two days (Fig. 1 A). They were similar to those observed in Clathrina cell cultures, but they were smaller, reaching only 43 µm diameter, and their filopodia were thinner and longer (Fig. 1 B). They moved, fed and replicated as Clathrina cells. Cultured sponge cells occasionally formed small clusters of up to 10 cells. These were not stable, and the actively moving cells separated rapidly from the cluster. Cells never formed contiguous monolayers, displaying a typical behavior of “amebocytes” as defined by Willmer (11,12).

Attempts to culture sponge cells in a defined nutritive liquid culture medium were not successful. Although cells could survive for more than one week in Dulbecco’s medium, their number progressively decreased, independently of serum addition. Rasmont (10), has also observed that freshwater sponges could not survive in liquid nutrient media, and needed bacteria for their alimentation. It may be argued that in Clathrina, the internal milieu is not well defined, since in this asconoid sponge, we do not know to what extent the choanoderm and pinacoderm exclude the direct contact of the very thin mesohyl with the sea water. In this case, amebocytes wandering in the mesohyl would depend upon their scavenger activity, and should not be necessarily equipped with transmembrane transport of organic molecules supplied by other cells. However, the internal milieu is controlled in Demosponges. In Polymastia, a single cell type “glyecocyte”, is in charge of both glycogen and lipid storage (5). This involves the systemic distribution, accumulation and subsequent use of energy reserves, stored in a single cell type, and poses the interesting question of their regulation. The incapacity of Polymastia cells to live on soluble molecules is thus surpris-
ing, and this may either be particular to the amebocyte cell type, or reflects the inadequate composition of the nutrient culture medium.

Although many cultures were accidentally lost in different stages of this study, we have maintained until now continuous replicating cultures of Polymastia cells for more than two years, without loss of viability. This is in agreement with the longevity of this species in nature. Conversely, Clathrina cell cultures were generally established in spring or summer, when these sponges are large and abundant in Rio de Janeiro. These cells proliferated actively until winter, but in the two years covered in this study, Clathrina cells decreased progressively their proliferative capacity in winter, their cultures entered the stationary phase of growth and eventually died out. This is in agreement with the seasonal distribution of Clathrina, abundant in summer, but practically absent in winter.

It is notable that both sponge cell cultures, a single cell type was obtained, bearing amebocyte characteristics, and we have never obtained the reconstitution of functional sponges. The major methodological difference among the cultures described in the present study and earlier works is the relative density of initial cell suspensions. High density cultures pass through formation of a thick continuous layer of dissociated cells at the bottom of culture flasks, containing different cell types together with extracellular matrix or adhesive molecules. This layer retracts and forms massive spheroids. These spherical structures were described as "diamorphs": they are equivalent to early post-larval stages, able to support a full reorganization and regeneration of a functional sponge (3). In the present cultures, extensively washed and diluted cells were cultured individually. Apparently, only the ameboid cell line was selected by this method.

Following the general definition of cell types given by Willmer (11,12), particularly well suited for sponge cell classification (3), amebocytes are cells that show no tendency to associate with other cells, nor to form integrated structures. They move freely, and are metabolically independent, able to phagocyte and digest extracellular materials. As such, they bear more characteristics of unicellular organisms than of cells belonging to a multicellular one. Although they move freely in the sponge mesohyl, amebocytes are always only a minor cell population. Their number is possibly controlled in the sponge body by a yet unknown molecular mechanisms, that apparently can be studied also in vitro. Recent demonstration of sponge cells proliferation sensitivity to controls involved in vertebrate cells (9) could indicate a very early evolution of such molecular mechanisms in multicellular organisms.

REFERENCES


Fig. 1. A, Polymastia ameboid cells on the second day of culture (×1680). B, Established culture of Polymastia amebocytes (×1280). C, Clathrina cells on the first day of culture (×1600). D, Clathrina ameboid cells on the second day of culture (×2000). E, Division of an amebocyte in an established culture of Clathrina cells (×1050).