Cytochalasin B Prevents Specific Sorting of Reaggregating Embryonic Cells

Abstract. *The effect of cytochalasin B on specific sorting during reaggregation of embryonic chick heart and neural retina cells was studied. At a dose that did not measurably affect uptake of precursors of protein and RNA synthesis, ratios of potassium to sodium ions, and nonspecific aggregation, cytochalasin B disrupted the formation of the characteristic pattern of islands of heart cells within a retinal continuum.*

Cells isolated from many embryonic tissues form characteristic aggregates when cultured in gyrotory shakers by the technique of Moscona (*1*). When two cell types are cocultured in this manner, they usually sort out from each other and form predictable patterns, with one cell type assuming a generally internal position, the other an external one. Although many mechanisms have been proposed to account for this specificity in reaggregation (*2*), its molecular basis is not known. However, the cellular rearrangement that occurs in this process is dependent, at least in part, on the activity of cell surface components (*3*). Cytochalasin B affects many different cellular events (*4, 5*), many of which may depend on the activity of contractile elements in the cell periphery (*6*). Therefore, it is of interest to determine the effects of cytochalasin B on the specific sorting of a reaggregating system.

Heart and neural retina cells from 10- or 11-day chick embryos were isolated with the use of crystallized trypsin (*7*) and were cultured in Eagle's minimum essential medium supplemented with 10 percent horse serum, 2 percent chick embryo extract, and 1 percent of a 200 m*M* solution of L-glutamine. Three-milliliter portions of cell suspension, containing 2×10^6 retinal cells and 0.5×10^6 heart cells per milliliter, were put into 25-ml stoppered flasks, and the flasks were placed in a gyrotory shaker with 2.5-cm radius of rotation, operating at 70 cycle/min at 37°C. More than 95 percent of neural retina cells and 90 percent of heart cells were viable, as assessed by trypan blue staining at the start of the experiment; the suspension was consistently unicellular. Cytochalasin B (Imperial Chemical Industries), 1 mg/ml in dimethylsulfoxide (DMSO), was added to flasks of aggregating cells to give final concentrations of 10, 5, 1, 0.5, and 0.1 μg/ml. Control cultures received equivalent amounts of DMSO. The aggregates were fixed in formalin after 72 hours of incubation, and the results are based on five separate experiments.

Figure 1 shows sections of fixed aggregates stained with periodic acid–Schiff reagent (PAS). Tissues that are PAS-positive stain bright red, whereas nonreactive tissues stain blue. It is difficult to make this difference clear in black and white photographs, particularly when the cells are intermixed, because the color difference shows up as only a variation in brightness. In aggregates from control cultures without DMSO or cytochalasin B (Fig. 1A), the PAS-positive (dark) heart cells showed the characteristic internal position in relation to PAS-negative neural retina cells, which are relatively light. Cells treated with 1 percent DMSO (by volume), a concentration equivalent to the 10 μg/ml cytochalasin B dose, showed some cell damage, although the sorting pattern remained evident (Fig. 1B). Cells treated with lower doses of DMSO were indistinguishable from untreated controls.

With cytochalasin B doses of 5 μg/ml (Fig. 1C) and 10 μg/ml, no sorting

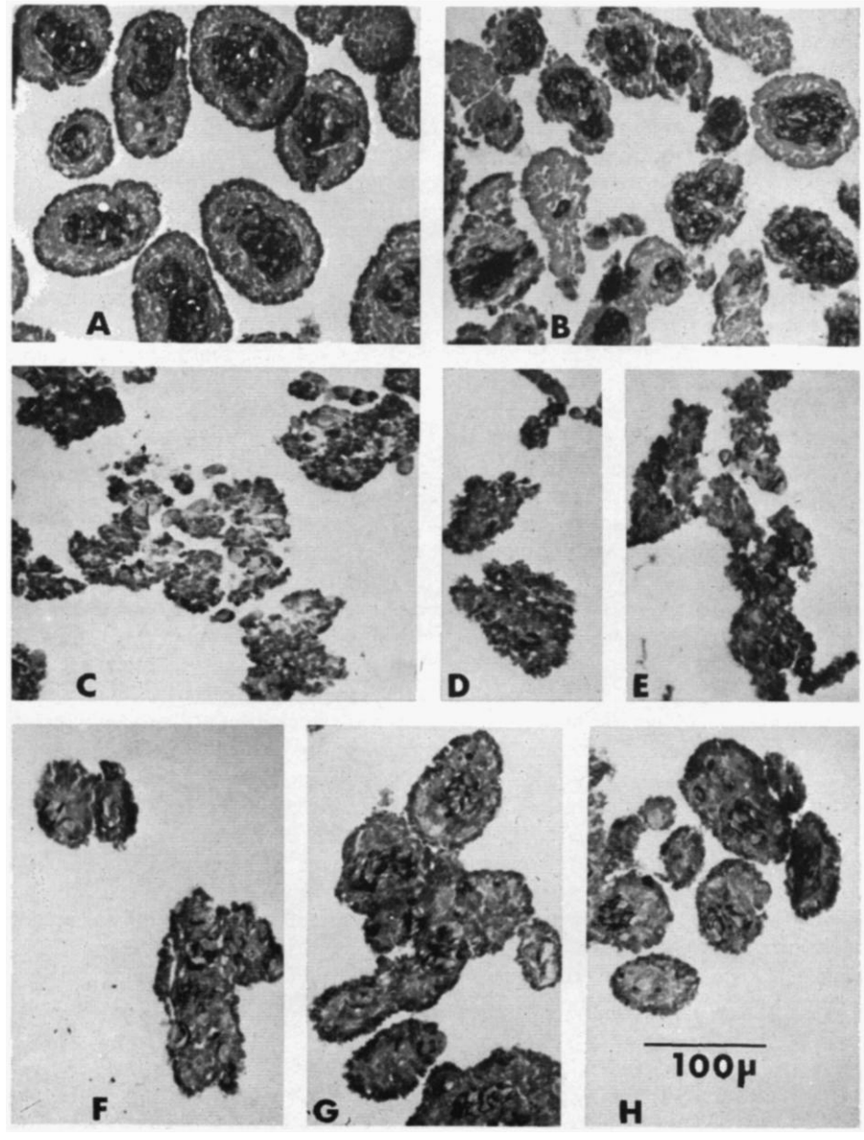

Fig. 1. Aggregates after 72 hours of culture; PAS stain. The cells staining lighter are from neural retina and the darker ones are from heart. Preparations are (A) control, (B) DMSO control (1 percent, by volume), and cytochalasin B at concentrations of (C) 5 μg/ml, (D and E) 1 μg/ml, (F) 0.5 μg/ml, and (G and H) 0.1 μg/ml.

Table 1. Uptake of protein and RNA precursors and K^+/Na^+ ratios in dissociated embryonic chick cells treated for 24 hours with cytochalasin B. Incubation volumes were 10 ml. In the incorporation experiments, neural retina cells were incubated with either [^{14}C]uridine (0.1 μc/ml) or [^{14}C]amino acid mixture (8 μc/ml), and the radioactivity of material precipitated by trichloroacetic acid (TCA) at 4°C was counted. In the cation ratio experiments, 1×10^7 heart cells and 2×10^7 neural retina cells were washed with isoosmotic choline chloride, and K^+ and Na^+ contents were measured with a flame photometer. A decrease in the K^+/Na^+ ratio under isoosmotic conditions indicates a loss of cellular K^+ coupled with a gain of Na^+. Healthy cells maintain a high K^+/Na^+ ratio. These results are the means of duplicate experiments.

	K^+/Na^+ ratio		Radioactivity in TCA precipitate (2×10^7 cells)			
			[^{14}C]Uridine		[^{14}C]Amino acids	
	Heart	Neural retina	1 hour	2 hours	1 hour	2 hours
DMSO control (0.5 percent)	2.2	4.4	6,580	16,990	17,520	25,150
Cytochalasin B (1 μg/ml)	1.8	4.1	7,830	12,180	15,860	30,770
Cytochalasin B (0.2 μg/ml)	1.9	4.3	7,370	12,930	19,270	31,590

was visible in any experiment. The groups of cells did not form characteristic aggregates but were often in loose clumps. The morphology of these cells indicated that many were dead or dying. With cytochalasin B at 1 μg/ml (Fig. 1, D and E) some aggregation took place in all experiments, but the heart cells did not take up an internal position with respect to neural retina cells and were distributed randomly in the aggregate. The morphology of cells treated with this and lower doses indicated that most cells were viable. With cytochalasin B at 0.1 μg/ml (Fig. 1, G and H), the aggregates were almost the same as control aggregates in most experiments; sorting occurred, but it was not as regular as in the controls. Aggregates formed in the presence of a 0.5 μg/ml dose of cytochalasin B (Fig. 1F) were intermediate between those formed at doses of 0.1 and 1 μg/ml. At the 0.5 μg/ml dose the results were less reproducible; perhaps this is the threshold dose for an effect on cell sorting specificity. In parallel experiments, cytochalasin B at 1 μg/ml did not prevent aggregate formation of neural retina or heart cells cultured separately.

Because cytochalasin B at 1 μg/ml reproducibly inhibited the normal sorting behavior, we investigated the effect of this concentration on three metabolic indicators of the condition of cell function. Heart and neural retina cells were cultured separately for 24 hours in complete medium with cytochalasin B (1 μg/ml) at a gyration speed (120 cycle/min) at which the cells did not aggregate. The rate of incorporation of [^{14}C]uridine and a mixture of [^{14}C]-amino acids into material precipitated by 10 percent trichloroacetic acid at 4°C was the same in these cells as in the controls (Table 1). We do not consider significant the differences shown in Table 1 for uridine and amino acid incorporation after 2 hours, although a decrease in uridine uptake has been reported for Novikoff hepatoma cells at cytochalasin B doses of 1 μg/ml (*8*). The intracellular Na^+ and K^+ concentrations of these cells were also measured (Table 1). Cytochalasin B did not significantly decrease the K^+/Na^+ ratio at the doses used (the K^+/Na^+ ratio in control heart cells was consistently less than that of control retinal cells). Therefore, cytochalasin B at a concentration that inhibited specific sorting behavior did not measurably affect uptake of protein or RNA precursors or the intracellular K^+/Na^+ ratio. Thus, the effects of cytochalasin B on the sorting behavior are not due to nonspecific cell-damaging effects.

Cytochalasin B disturbs morphogenesis in explants from different tissues (*9*), nonspecific aggregation of chondrogenic and myogenic cells (*10*), adhesion of tumor cells to glass substrates (*11*), and cell movements (*5*). Although the detailed mechanism of its action is not known, cytochalasin B has been thought to affect these processes by disrupting contractile elements in the cell periphery (*6*). Thus, cytochalasin B could act to prevent specific sorting by disrupting contractile elements in the cell periphery and subsequently inhibiting movement of cells within the aggregate. Alternatively, cytochalasin B could specifically inhibit the formation of cell surface moieties involved in specific recognition processes or movement or both these activities. Sanger and Holtzer (*10*) have reported that low doses of cytochalasin B inhibit incorporation of glucosamine into mucopolysaccharides. These substances, which are found at cell surfaces, may contribute to nonspecific aggregation (*12*), but they have not been shown to be involved in specific sorting. It has not been resolved whether actin filaments and actomycin are sensitive to cytochalasin (*13, 14*), and caution should be taken in extrapolating results obtained with muscle cells (*10*) to all cells. The results in this report emphasize that the specific sorting of cells may result from different mechanisms from those involved in the initial formation of aggregates.

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