REGULATION OF RNA SYNTHESIS THROUGH CELL CONTACT IN SPONGE CELLS

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ABSTRACT

Evidence of cell contact regulating RNA synthesis in sponge cells has been recorded. A steady increase in RNA synthesis was observable during aggregation of these cells, but in cells which were artificially maintained nonadhering in a dilute solution of methyl cellulose, the incorporation of ^{3}H-uracil into TCA precipitate was extensively enhanced. When the non-contacting cells were made to contact by washing away methyl cellulose, a suppression of this magnified synthesis was brought in. This depicts a morphogenetic peculiarity of sponge cells. The cells appear to have dual individuality, *viz.*, as single entities and as components of a cooperative multicellular system.

INTRODUCTION

COMPLEX is the process of pattern formation where each of the cells involved has to recognise its position and take upon the path of ultimate differentiation. The role of cell surface as a mediator of cell interaction and in transmitting the signals to the interior of the cell has clearly been enunciated (Nicolson[1]). Thus it is clear that communication between cells mitigates some control in regulation of growth, development and maintenance of steady state of shape of an organism. Cell communication seems to constitute both long range and short range interactions, the short range interactions requiring direct contact between cells (Gilula[2]). The control of one cell on another's metabolic state is well exemplified by contact inhibition of proliferation in culture cells (Stocker and Rubin[3]) and cooperation of cells by transfer of molecules has been depicted in many instances (Suback-Sharpe *et al.*,[4] Cox *et al.*[5]).

Dissociated sponge cells which aggregate to reform the original pattern (Wilson[6]) can be an ideal material for studying the characteristics of cooperation and communication during morphogenesis. Here the ability of contact and adhesion are innate morphogenetic parameters special to each cell and without the specific cell to cell recognition, the identity of the restituted sponge will not be feasible. This situation has been taken as a criterion for understanding the process of cell communication in controlling the cellular synthetic processes through contact. We report here some of the characteristics of isolated cells in situations where they were engineered in such a way as not to have direct communication after experimental dissociation. The present experiment outlines how in such an altered situation, where cells are mechanically prevented from forming cell contacts and establishing a consolidated cellular pattern, the spectrum of RNA synthesis becomes altered.

MATERIALS AND METHODS

Fresh water sponge *Spongilla carteri* was collected from a local pond and kept in running water. The tissue within two days of collection was always used.

Pieces of sponges were freed from small stones and cut into 1 cm^{3} pieces. The tissue was washed in millipore filtered pond water and the pieces placed in between the folds of fine silk were squeezed into a beaker containing the medium. Other procedures for cell separation and culture were as described previously (Kartha and Mookerjee[7]). The aggregates could undergo differentiation and reform miniature sponges in about 48 hours.

In order to keep the cells separate and noncontacting, a technique has been evolved using a mesh of methyl cellulose, a long polymer in which the cells were kept nonmoving and noncontacting. A 1·5% solution of recrystallized methyl cellulose (Blenkinsop and Co., London) was layered in 10 cm diameter petri dishes and 3 ml each of cell suspension was carefully poured over the methyl cellulose layer. The aggregating cells were incubated in 4 cm diameter petri dishes and as the cells were self-aggregating the use of a shaker was not necessary.

RNA synthesis in aggregating and non-aggregating cells was measured by the incorporation of ^{3}H-uracil (1 μ Ci/ml) BARC, Bombay, Sp. act. 49·3 mCi/mmole) into the material precipitable by trichloroacetic acid (TCA). After 1 hour of labelling as indicated below the cells and aggregates were washed off methyl cellulose, homogenized in the cold and the nucleic acids were precipitated by 5% TCA. The precipitate retained on millipore filters (Millipore Corporation, U.S.A.) was washed several times with cold 5% TCA and once with absolute ethanol-ether (3 : 1). The filters were dried and radioactivity was determined in a Packard Tricarb Scintillation counter-meter using toluene based scintillant with PPO and POPOP.

RESULTS

Figure 1 shows that the incorporation of uracil maintains a steady state of increase when the cells were aggregating. The cells were pulse labelled for 1 hour at 0, 1, 3 and 5 hours after dissociation and TCA precipitate was extracted consecutively.

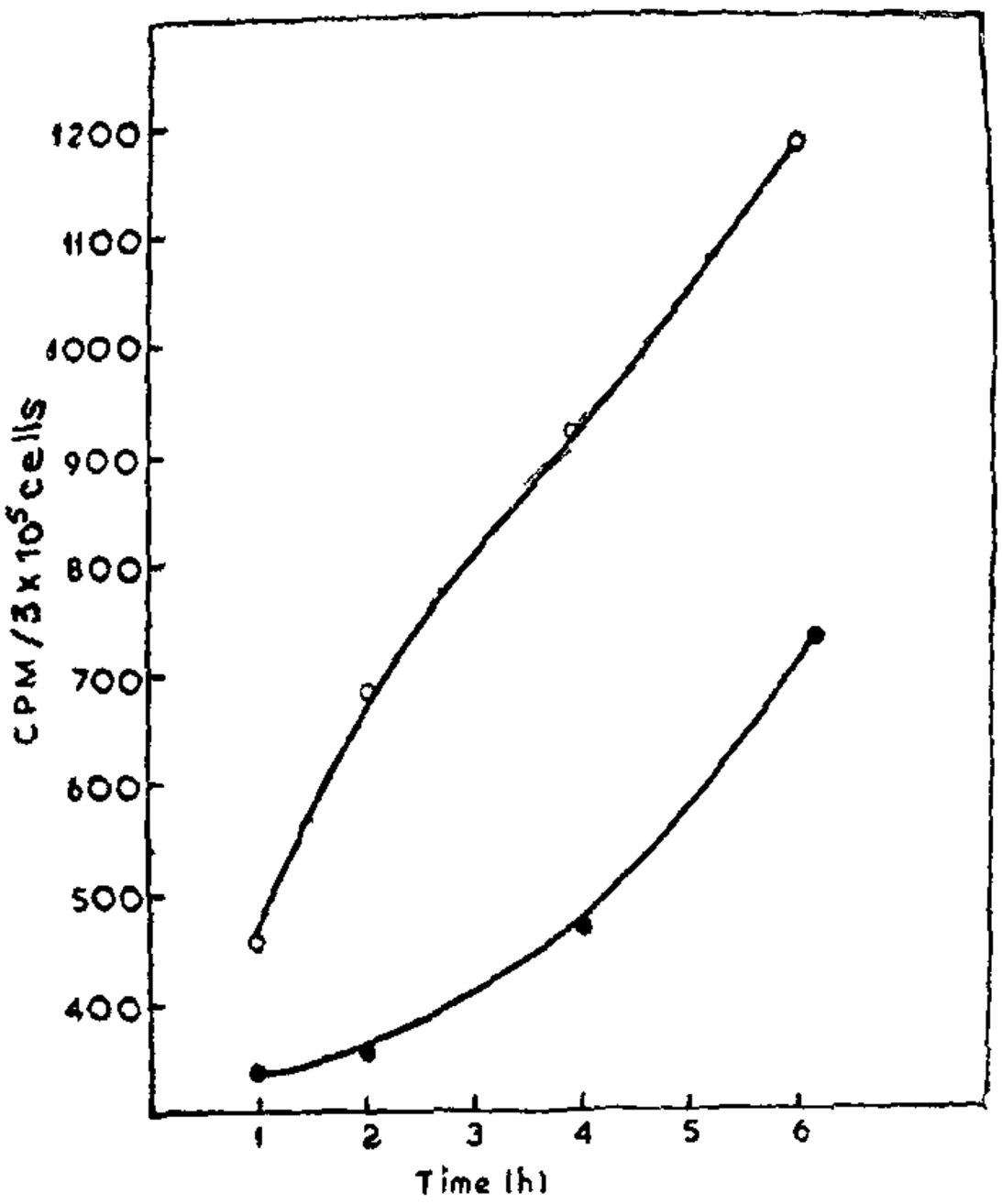

FIG. 1. Synthesis of RNA in contacting and noncontacting cells. 5 ml of cell suspension containing 2×10^5/ml cells were cultured with or without methyl-cellulose and pulse labelled with 1·5 μ Ci/ml of H^3-uracil. ●——● contacting cells, ○——○ noncontacting single cells.

The cells of the sponge aggregate almost immediately after the act of dissociation. The behaviour of the cells in relation to RNA synthesis was followed in order to trace the relationship between adhesion and macromolecular synthesis. A method has been devised to maintain the cells in separate and noncontacting state, but viable, in which the polymer methyl cellulose was used as the microbarrier between the cells, which were kept apart in the mesh. The cells were layered over a 1·0% solution of methyl cellulose and the incorporation of ^{3}H-uracil was noted for a period of 6 hours. In these noncontacting cells, the pattern of synthesis of RNA showed a separate turn where the rate of incorporation is accelerated (Fig. 1) deviating from the pattern in the aggregating cells.

In order to exclude the possibilities of differential uptake, a pool of uracil was maintained in the tissue before dissociation and the suspension was set up for the experiment without the addition of ^{3}H-uracil later. The cells of aggregating and nonaggregating culture were derived from the same tissue in which the RNA precursor pool size was identical. It is evident from Fig. 2 that from this pool of precursors, the mode of RNA synthesis was different for the cells in different conditions, *viz.*, contacting and noncontacting.

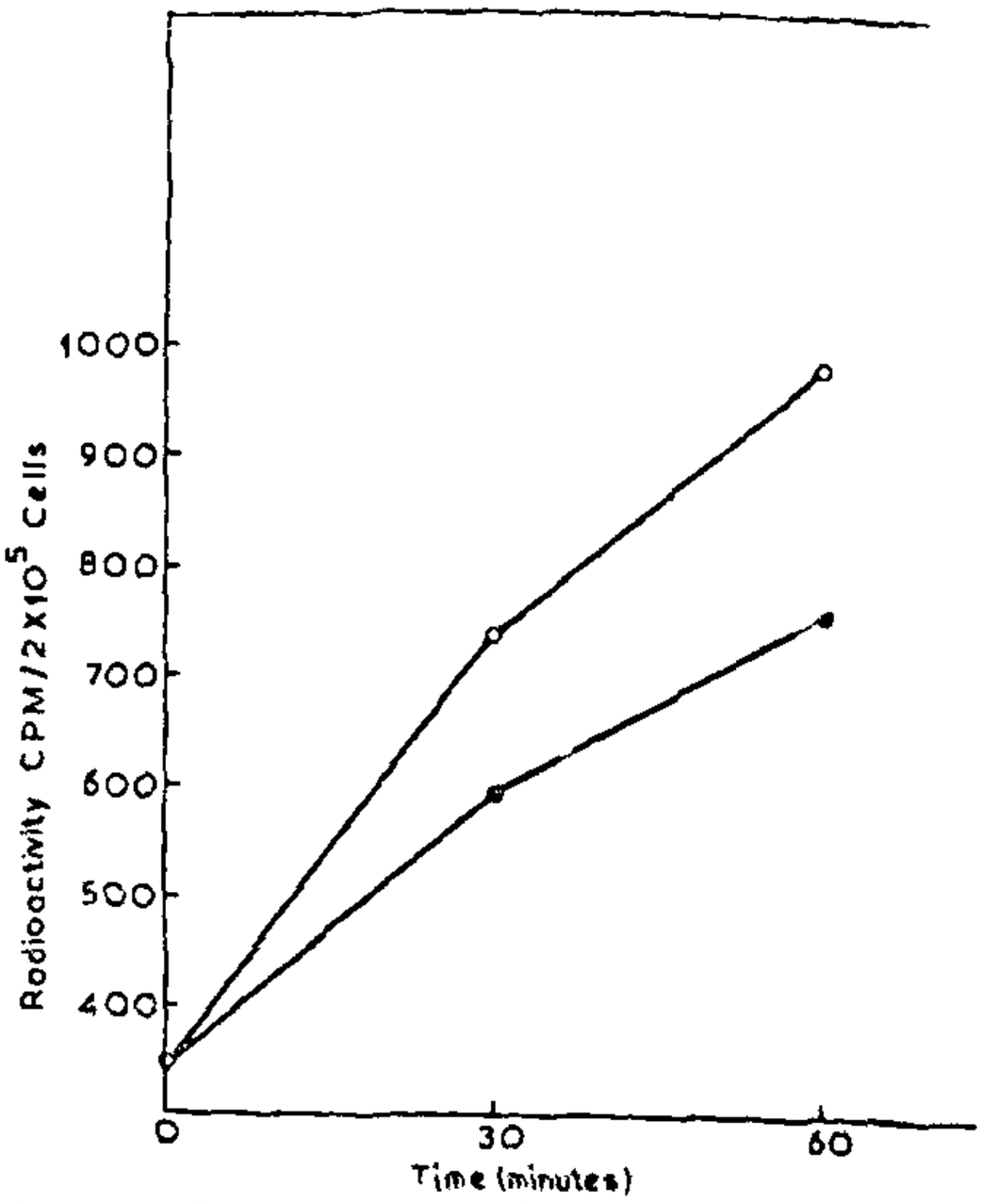

FIG. 2. Incorporation of ^{3}H-uracil into TCA precipitable material in contacting and noncontacting cells. The intact tissue was labelled for 1 hr with ^{3}H-uracil (1·5 μ Ci/ml) and after dissociation the cells for aggregating (○——○) and nonaggregating (●——●) set ups were cultured in isotope-free medium.

If the stimulated synthesis was because of lack of contact, the initiation or reestablishment of the contact between the cells should affect inversely the synthesis of RNA. To test this assumption, a dilute-suspension of noncontacting cells in methyl cellulose was brought together by removing the mechanical molecular barrier. The cells in methyl cellulose were washed and cultured in normal medium where the cells immediately display the property of aggregation. The stimulated synthesis as shown in their noncontacting disposition could no more be observed and the total RNA synthesis showed a declining trend towards that of the normally aggregating cells (Fig. 3). The variations in all these experimental results were found to be less than 5%.

DISCUSSION

The experiments have clearly shown that cell contact exerts a kind of signal in regulating cellular synthetic processes, especially RNA turnover. During the dissociation and aggregation, when the original tissue pattern is decapacitated, the cells begin the synthesis of informative molecules, the regulation being the communication between cells. But when the cells are discrete and remain separate, this system of regulation is kept in abeyance and the individual

cells, in the absence of apt communication with other cells harness the synthesis of RNA in an elaborate way. Once the contact is mechanically reestablished, a kind of suppression of RNA synthetic machinery takes place.

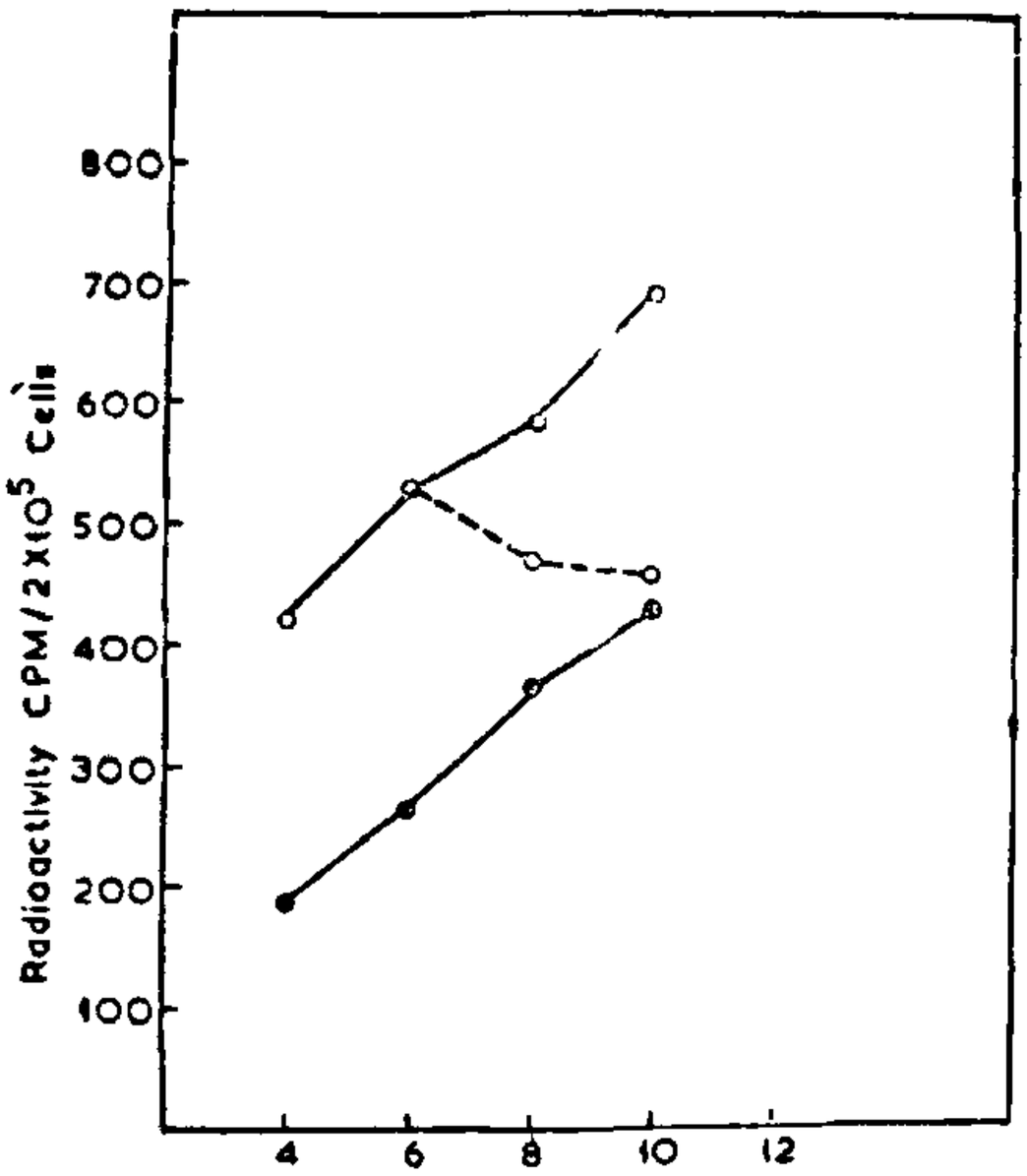

FIG. 3. Inhibition of RNA synthesis when noncontacting cells were brought together and made to aggregate. ●——● aggregating cells, ○——○ non-aggregating cells, ○——○ noncontacting cells made to aggregate.

In other developmental systems, the programme of metabolic pattern is found to be dependent on proper organisation of cells. In dissociated sea urchin gastrula cells, which have been kept noncontacting, the synthesis of RNA is slackened (Giudice *et al.*[8]) along with a decrease in the activities of enzymes like DNA polymerase, thymidine kinase, etc. (De Petrocellis and Vittorelli[9]). Similarly, only aggregating cells of Xenopus (Abe *et al.*[10]) and chick neural retina (Morris and Moscona[11]) have a higher alkaline phosphatase activity or glutamine synthetase induction respectively. Although the mechanisms of these cell contacts which are dependent on regulatory system are not clear, indications are there that the direct interaction of surface membrane molecules are involved in these reactions. Muller *et al.*[12] could find from their experiments on marine sponge *Geodia* that the introduction of purified aggregation factor to the cells which were washed off this factor from the cell surface could enhance the synthesis of DNA, RNA and protein along with the initiation of mitosis. Levine *et al.*[13] showed that the addition of the purified surface membrane to the cultured cells could bring in cessation of polyribosome synthesis associated with contact inhibition of proliferation. It is also suggestive that the physical contact between surface membranes rather than diffusable factors may relay the information for such control.

The data obtained in the present experiment depict a peculiar morphogenetic speciality belonging to sponge cells. Sponge tissue has been described as a cellular republic, assigning special individuality to each of the cell types. The capability for dual existence has been indicated in the transformative steps of sponge cells (Kartha and Mookerjee[14], Mookerjee and Makhija[15]). When the cells are separate and noncontacting, they are prone to behave as single cells and synthesise the necessary macromolecules for their separate existence. Here the archaic protozoan characters may be displayed. Experimentally, it has been shown that some of the dissociated sponge cells have a free state of existence (Mookerjee *et al.*[16]). But when they are in ensemble, this isolated luxury is not called for as it is a cooperative system of coexistence of cells.

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