filled with the fine collagen fibrils. This fine collagenous material is also interposed between these cells and the lining layer, and it contrasts sharply with the fibrous bundles of the surrounding stroma. Fenestrated capillaries are often observed near these dense granule-containing cells.

These cells, which correspond in position to the B cells described in other species, show, in the mouse, evidence of active secretory function. Their rough endoplasmic reticulum is well developed, as is the Golgi apparatus, which shows classic images of elaboration of secretion granules: budding off of vesicles which then increase in density to give rise to dense secretory granules, with a mean diameter of 200 nm (max. 220 nm) (figures 1 and 2). It has not yet been possible clearly to visualize the secretory material on histological sections. At best, can one detect a fine intracytoplasmic granularity weakly colored with PAS on semi-thin sections. An absence of metachromasy with toluidine blue and a negative reaction with alcian blue pH 2.4 clearly distinguishes these cells from mastocytes, which are strongly colored by these 2 techniques.

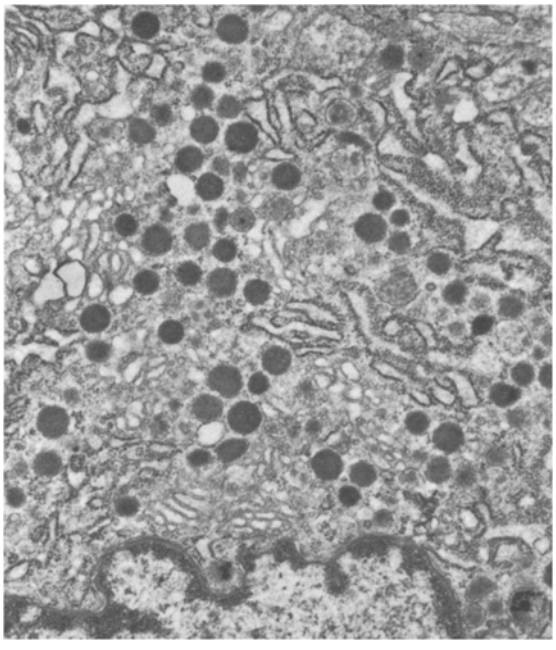

Fig. 2. Detail of a B cell showing the well developed rough endoplasmic reticulum, dense secretory granules and formation of granules by the Golgi complex. ×14,300.

Besides the elaboration of secretory granules, presumably proteins, these cells appear to have a fibroblastic activity, as evidenced by the abundance of fine collagen fibrils which lie around them and even within surface invaginations. The specific secretory activity of these connective tissue cells is probably responsible for the modification of their surrounding stroma. One can only conjuncture regarding the role of the elaborated material. It should be remembered, for example, that hyaluronic acid in synovial fluid is bound to a special protein, whose origin is yet undetermined[9,10]. Also a 'connective tissue activating peptide' has recently been isolated from synovial tissue[11]. In addition, the possibility that these cells are involved in a hormonal regulatory mechanism should be kept open, considering the frequent proximity of B cells and underlying fenestrated capillaries. In any case, it is obvious that these cells play an important and probably specific role in the metabolism of the connective tissue associated with the synovial cavity. The synovial membrane of the mouse provides a particularly useful model for further investigations of function of joint connective tissues, utilizing histochemic and radioautographic techniques.

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The effect of concanavalin-A on the reaggregation of cells dissociated from Xenopus laevis early embryos

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Summary. The effects of concanavalin-A on the reaggregation and sorting of cells from Xenopus laevis early embryos have been studied. The results suggest that at high concentrations, concanavalin-A can prevent reaggregation.

Concanavalin-A (Con-A) is a plant lectin which binds preferentially to α-methyl-mannoside and α-methyl-D-glucopyranoside carbohydrate residues[1], and thus it can be used to block these groups on cell surfaces. Such groups could play a part in the control of morphogenesis, since Con-A has been shown to affect a number of embryonic systems such as sea urchin embryos[2] and chick retinal cells[3]. The results of a number of recent experiments suggest that Con-A binding residues might have a role in amphibian early morphogenesis too. Embryos of Amblystoma maculatum cultured in Con-A show a slower rate of development than normal, and are blocked at gastrulation[4]. If embryos of Xenopus laevis are exposed to fluorescein-isothiocyanate-labelled Con-A to localize the binding sites, concentrations of label are seen at the dorsal lip of the blastopore of the gastrula and on the neural folds of the neurula[5], both regions associated with active morphogenetic movements. Fluorescein-labelled Con-A has also been used to demonstrate a change in membrane properties of amphibian cells at gastrulation: isolated Rana pipiens blastula cells bind Con-A

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uniformly over their surface, whereas isolated gastrula cells form a cap of binding sites at one end of the cell, suggesting that these cells have a more fluid membrane[6]. It is well established that cells dissociated from amphibian early embryos can reaggregate and sort out according to tissue type[7]. Since the cells of hybrid amphibian embryos which arrest at gastrulation have a reduced adhesion[8], it is possible that the factors which control reaggregation and sorting are the same as those which control normal morphogenesis. Therefore one way of testing the possible importance of Con-A binding groups in the control of amphibian morphogenesis might be to test the effect of Con-A on reaggregation and sorting. Here we describe the preliminary results of some such experiments.

Materials and methods. Xenopus embryos were obtained by injecting adult frogs with chorionic gonadotrophin (Chorulon, Intervet Ltd.) and the jelly was removed chemically[9]. The medium used for the culture of cells was Stearn's medium[10], pH 7.3. Blastulae (stage 8–9[11]), gastrulae (stage $10^1/_2$[11]) and neurulae (stage 18–19[11]) were dissociated within their vitelline membranes in calcium- and magnesium-free medium with 4 mM EDTA for 2 or 3 h. The culture chambers were small plastic petri dishes to which had been added 8 ml of 1% ionagar in medium plus 0.1 mg/ml of Streptomycin (Glaxo Labs. Ltd.) and 25 IU/ml of Penicillin (Glaxo Labs. Ltd.). Small wells were made in the agar with a heated pyrex glass rod, and 5 ml of culture medium were added to each dish. The culture medium was medium plus antibiotics plus 3% horse serum (Gibco-Biocult) to which various concentrations of Con-A (Sigma Ltd.) were added.

The dissociated embryos were transferred to the culture wells, and the vitelline membranes were removed using watchmakers' forceps. The cells were completely separated by carefully taking them up into a micropipette, and expelling them into the well. They were cultured at 20 ± 0.5 °C and control cultures were observed at 24, 48 and 72 h. The effects of Con-A treatment were scored at 24 h, and some aggregates were fixed in Smith's formal-bichromate fixative for routine histology. Scanning electron microscopical observations on Xenopus cell aggregates will be described elsewhere[12].

Results and discussion. Control cultures: After 24 h, blastula cells had formed single large spheroidal aggregates consisting of a core of larger non-pigmented endoderm cells covered with patches of pigmented ectoderm cells. Under the conditions used, the majority of cells in each well appeared to be incorporated into the aggregate. After 48 h, the ectoderm had formed fewer larger patches, and after 72 h the ectoderm cells were becoming translucent, indicating that they were differentiating[13]. Gastrula cells formed similar aggregates but in the case of neurula cells only non-pigmented cells were incorporated into the aggregate.

Con-A treated cultures: Preliminary experiments were conducted with blastula cells, since the mixed aggregates formed allowed the effects of Con-A on cell sorting, as well as aggregation, to be studied. The results of these experiments suggested that low concentrations of Con-A (25 μg/ml and 50 μg/ml) had no effect on either cell aggregation or cell sorting, despite the fact that at these concentrations Con-A binds at detectable levels to dissociated amphibian cells[6]. Following this finding, a range of higher concentrations (125 μg/ml, 250 μg/ml and 500 μg/ml), at which Con-A is known to affect amphibian development[4], were used.

At each of these higher concentrations, Con-A caused a loose agglutination of cells. Where reaggregation was inhibited, the cells in the well appeared loosely adherent, in contrast to their appearance immediately after dissociation: when aggregates were present they were covered with loosely adherent cells which were removed by handling. This loose agglutination of cells was prevented by the inclusion of the appropriate hapten, 0.1 M α-methyl-mannoside (Sigma Ltd.), in the culture medium, except at the highest concentration of Con-A (500 μg/ml) where some agglutination was observed. The agglutination of cells by Con-A is consistent with the observation that Con-A agglutinates sea urchin embryonic cells[14]. Although not all cells of amphibian intact embryos bind Con-A[5], Con-A binding sites are exposed by EDTA treatment[6]. The Con-A used in the present experiments was divalent, and presumably caused agglutination by linking sites on adjacent cells.

At higher concentrations Con-A appeared to inhibit amphibian cell aggregation (table), as it can inhibit reaggregation of sea urchin embryonic cells[2]. Some variability between different batches of embryos was evident: for example, in 2 experiments 250 μg/ml Con-A inhibited reaggregation of blastula cells, in 1 experiment it did not. When blastula and gastrula aggregates did form in the presence of Con-A, cell sorting occurred in the majority of cases. Unlike the effects of high concentrations on whole embryos[4], the inhibition of aggregation of gastrula cells by 500 μg/ml was prevented by 0.1 M α-methyl-mannoside. However, at higher concentrations some precipitation of Con-A occurred after 24 h, and although observations showed that reaggregation had started within 3 h of culture, it is possible that some inhibition of aggregation could be due to mechanical disruption. The results (table) might also suggest that there is a difference in the effect of Con-A at different stages of development, and further experiments with embryos from the same batch are being undertaken in an attempt to test this.

The cell surface appears to play a role in the control morphogenesis and in cell recognition[7], and any tool such as Con-A which binds to specific surface residues can be of value in the analysis of the molecular mechanisms of such phenomena.

Effect of concanavalin-A on the reaggregation of cells dissociated from early embryos of Xenopus laevis

Stage	Blastula	Gastrula	Neurula
Concentration Con-A (μg/ml)			
50	+		
125	±	+	±
250	±/−	+	±
500		−	−

+, normal aggregation; ±, fewer aggregates; −, no aggregates.

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