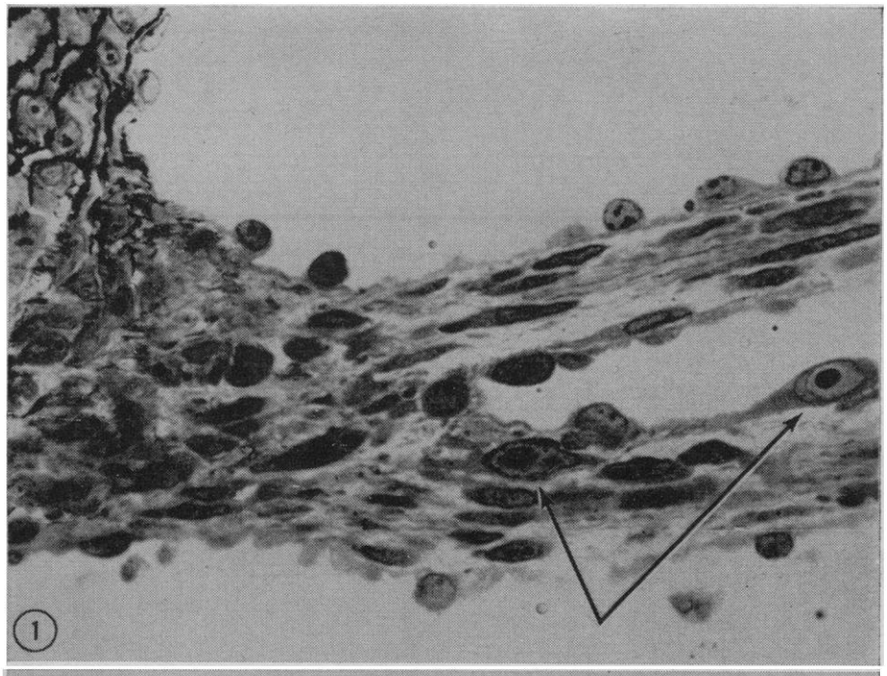
Also in these foci of necrosis were degenerating cells from which large masses of virions were being spilled into the interstitium of the lamina propria.

The reparative process in mice infected with murine adenovirus remains to be investigated. Presumably, acute lesions, such as we observed in the valves, may be expected to heal, with resultant scar formation and dystrophic calcification. Such chronic lesions were seen in the myocardium in this same experimental model.

Viruses are capable of invading and damaging cardiovascular tissues in man (*9*). The extent and incidence of such lesions, especially viral endocarditis, are not well defined. Our study and that of Burch *et al.* (*4*) make it appear that more than one virus may multiply in and destroy cells of cardiac valve tissue in experimental animals. Whether a similar phenomenon occurs in man is unknown. However, from changes in electrocardiogram it is well known that many generalized viral infections in man may be associated with myocardial involvement. This point raises the possibility that in at least some of these cases valvular involvement may occur, with subsequent scarring and resultant chronic valvular disease.



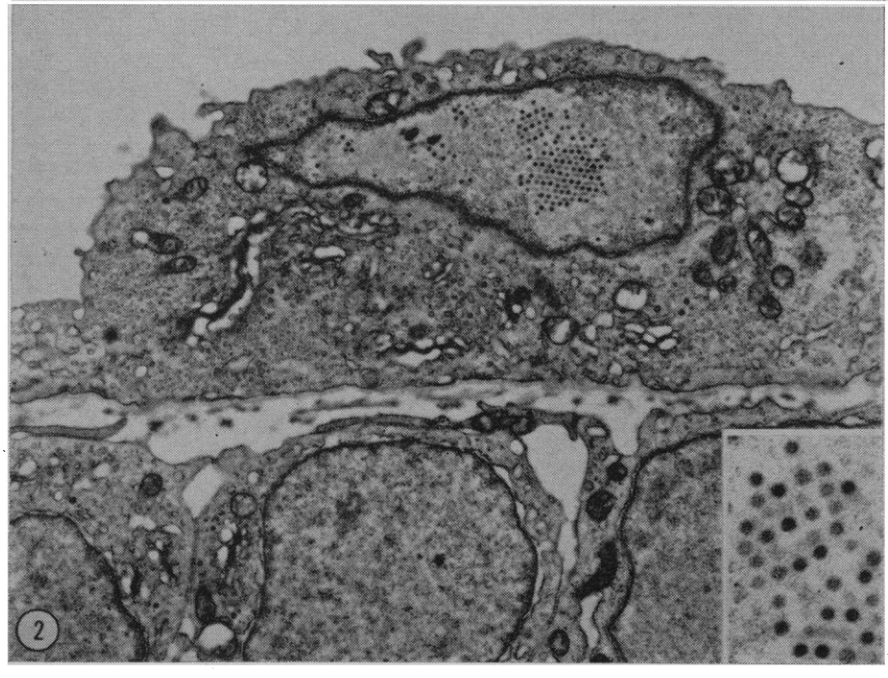


Fig. 1 (top). Commisure and leaflets of aortic valve from a mouse infected with adenovirus, showing inclusion bodies (arrows) within nuclei of endothelial cells and fibroblasts. One-micron section of araldite-embedded material; Paragon multiple stain; about × 600. Fig. 2 (bottom). Electron micrograph of valve lesion (Fig. 1); the nucleus of the endothelial cell contains adenovirions in crystalline arrays; about × 8300. Inset is a view of virions in detail; × 34,600.

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Cell Aggregation: Its Enhancement by a Supernatant from Cultures of Homologous Cells

Abstract. *A supernatant medium has been prepared from living embryonic neural retina cells which specifically promotes their histogenetic aggregation. Its function is dependent upon at least two experimentally separable steps: selective uptake and functional utilization.*

The mechanisms that mediate and control mutual attachment and histogenetic aggregation of embryonic cells are a major factor in considerations of differentiation and multicellular organization (*1*). Much information on this

has been obtained from studies on reaggregation of dissociated embryonic cells under controlled culture conditions in vitro and their ability to reconstruct tissues (*2*). On the basis of these studies it has been suggested, as a working concept, that histogenetic attachment of cells is mediated by specific cell products that function at the cell surface or between cells and that their molecular characteristics play a role in conferring upon embryonic cells the properties of "selective affinities" and "surface specificities" that are essential for histogenesis (*3*). The validity of this general concept was supported by the isolation and characterization of cell products which specifically enhance the aggregation of dissociated sponge cells (*4*). These materials were obtained by "washing" living cells, and the evidence suggested strongly that their enhancing effect on cell aggregation was due to their function at the cell surface and between cells. Concurrently, work in this laboratory on vertebrate embryonic cells demonstrated that it was equally feasible to obtain materials with specific aggregation-promoting activity from embryonic chick cells (*5*). Our report describes the preparation and some of the biological properties of a supernatant medium that enhances cell aggregation; it was obtained from living embryonic cells by a more efficient method than those used earlier (*5*).

Suspensions of neural retina cells from 10-day chick embryos were prepared by the standard procedure of dissociation with trypsin (*2*) (Worthington three times crystallized or Armour Tryptar). Monolayer cultures of these cells were set up in 60-mm Falcon dishes at a cell density of one 10-day retina (approximately 80×10^6 cells) in 3 ml of Eagle's basal medium (EM) with 20 percent fetal bovine serum (Microbiological Associates). After 48 hours the medium was decanted; the cultures were washed twice in Tyrode's solution and were supplied with fresh EM without serum. After further 24-hour incubation the medium was collected and cleared of cells and debris by centrifugation at 10,000 rev/min for 30 minutes; the supernatant was dialyzed against Tyrode's solution (10 ml of supernatant medium against two 1-liter changes of Tyrode's solution). This dialyzed supernatant medium was diluted with an equal volume of Tyrode's solution, and it will be referred to as $R^{10}S$. Its effect on aggregation of dissociated 10-day retina cells was assayed by the procedure of cell aggregation by rotation (*2*): the size of 24-hour aggregates obtained at 37°C in cultures with $R^{10}S$ was compared with that obtained in control medium (EM). As in previous work with media free of serum (*5*, *6*), deoxyribonuclease was added to all culture media (10 μg/ml) to prevent the formation of interfering viscous materials.

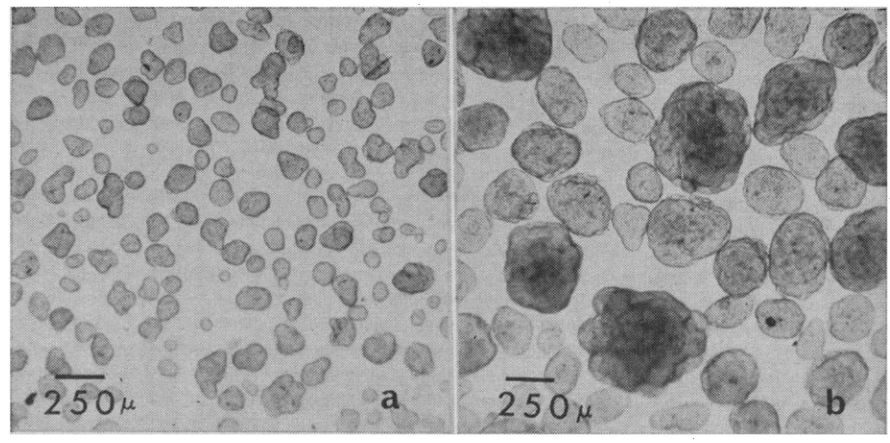

Fig. 1. (a) Aggregates of dissociated retina cells (from 10-day chick embryos) after 24 hours in control medium. (b) Aggregates of dissociated retina cells (from 10-day chick embryos) after 24 hours in medium ($R^{10}S$) that promotes aggregation.

The $R^{10}S$ supernatant medium prepared and tested as described above enhanced the aggregation of dissociated 10-day embryonic retina cells, as evidenced by the significantly larger size of the 24-hour aggregates compared to controls. Figure 1 shows the differences in the size of 24-hour aggregates of these cells obtained in control medium (Fig. 1a) and in $R^{10}S$ medium (Fig. 1b). Figure 2 summarizes the size distribution of aggregates in representative control and experimental cultures. These results were highly consistent and repeatable under the conditions described.

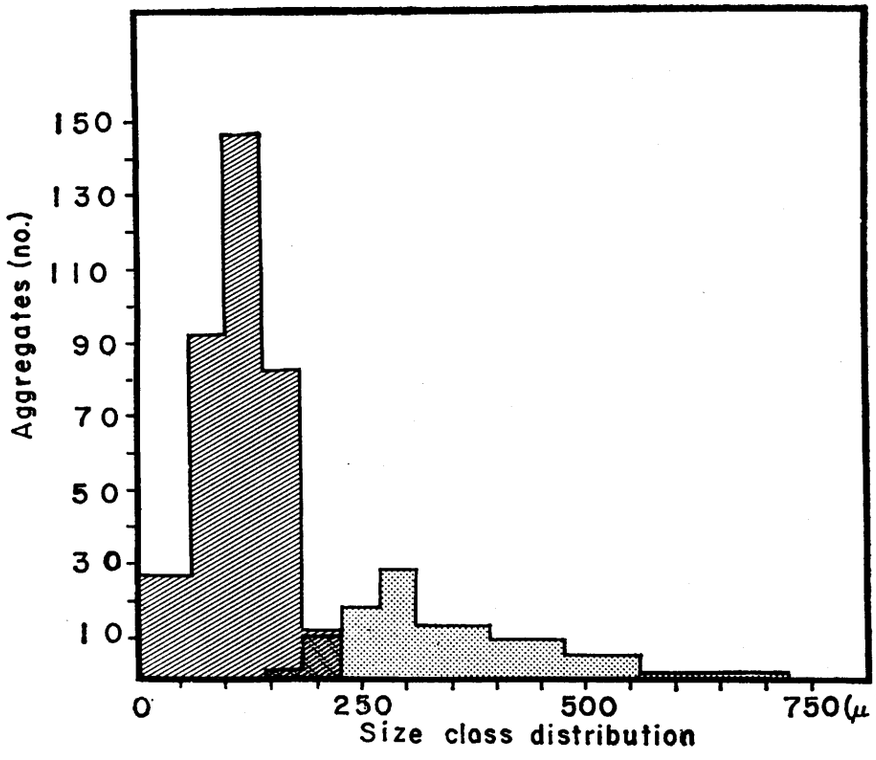

Fig. 2. The distribution of size classes of aggregates in control cultures (striped) and in medium that promotes aggregation (dotted). Aggregates were grouped into 12 size classes by comparison with circles of known size.

We tested the specificity of the $R^{10}S$ effect by determining whether it would similarly enhance the aggregation of other embryonic cells dissociated from 10-day liver, 6-day heart, and 4-day limb buds. In no case was there any demonstrable effect of $R^{10}S$ on the size of the aggregates of these cells. A supernatant medium was prepared (according to the procedure for $R^{10}S$) from monolayer cultures of 10-day embryonic liver cells; it had no demonstrable effect on the aggregation of 10-day embryonic retina cells.

Dialysis of the supernatant medium was essential for demonstration of the specificity of its effect. Thus, nondialyzed supernatants from monolayer cultures of liver, limb buds, heart, and retina cells enhanced the aggregation of 10-day embryonic liver cells. This nonspecific effect was found to be due to a serum component taken up by the cells during the first 48 hours in monolayer culture; it is then released into the serum-free medium during the 24-hour period preceding collection of the supernatant fluid. Dialysis removes or inactivates it.

Initial studies on the formation of the active constituent in $R^{10}S$ indicate that it does not accumulate in the culture medium if the monolayer cultures are kept at 4°C or are treated with cycloheximide (2 μg/ml) during the 24 hours preceding collection of the supernatant. This finding suggests that the formation or release of the active constituent by the cells requires biosynthetic processes involving protein synthesis. In this context it is of interest that low temperatures and inhibitors of protein synthesis also inhibit aggregation of embryonic cells dissociated with trypsin, presumably by interfering with the synthesis of constituents degraded by trypsin and required for continued aggregation of cells (*5*, *6*).

The enhancement of aggregation by $R^{10}S$ requires temperature-dependent processes in the responding cells. Thus, in cultures rotated for 24 hours at 4°C there is no effect; however, when these cultures are transferred to 38°C and further rotated for 24 hours, enhancement of aggregation occurs. Although at 4°C $R^{10}S$ had no visible effect, the active constituent was taken up by the cells. This was determined as follows. Dissociated 10-day retina cells dispersed in medium with $R^{10}S$ were maintained in rotation cultures at 4°C for 24 hours. The medium was then separated from the cells and tested on freshly dissociated 10-day retina cells at 38°C to determine whether it retained its typical activity. No measurable activity could be detected; this, together with the other results, strongly indicates that at 4°C the active constituent is taken up by the cells, but that its function in enhancing aggregation requires metabolic activity. Further evidence along this line is provided by the fact that if $R^{10}S$ is exposed at 4°C for 24 hours to 10-day embryonic liver cells, it does not appreciably lose its capacity to enhance the aggregation of retina cells at 38°C; this indicates that the $R^{10}S$ constituent is not taken up by liver cells to the same extent as it is by retina cells and further demonstrates its functional specificity. Thus, the enhancement of aggregation by $R^{10}S$ appears to involve two main phases, as suggested on the basis of earlier work (*5*): selective uptake and functional utilization. The mechanisms involved in these phases are being studied.

The active constituent of $R^{10}S$ is not destroyed by deoxyribonuclease or ribonuclease; it sediments by centrifugation overnight at 105,000*g*. This finding suggests that the active constituent has a particulate nature, as in the case of the factor which promotes aggregation of sponge cells (*4*). Our results raise questions concerning the characteristics of the active constituent in $R^{10}S$ and possible changes in its properties with cell differentiation; further studies should also determine whether the procedures used in its preparation and bioassay are effectively applicable to the isolation from other cells of materials which promote aggregation, or whether in other cases such materials are more strongly bound to the cells and not as readily obtainable in solution.

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Hematuria Following Administration of Ethanol

Abstract. *Hematuria, with some erythrocytes also appearing in the urine, was observed in male albino rats given 20 percent and 40 percent ethanol in lieu of drinking water for several weeks. However, the amounts of hemoglobin in the blood of the treated group did not change relative to those of the control group; this finding indicates that the degree of hematuria was not sufficient to produce an anemia.*

Although there have been numerous reports (*1*) describing the occurrence of hematuria in various pathological states, we are the first, to our knowledge, to describe hematuria following the administration of ethanol to an experimental animal.

The hematuria was first observed fortuitously after 15 weeks of administration of ethanol to experimental (male) Sprague-Dawley albino rats. The urine samples were observed to be darker in color than those of the control group given water. With respect to the amounts of urinary and fecal porphyrins, there was no significant difference between the two groups. This observation prompted the qualitative testing for the presence of blood in these urine samples by test papers (Hemastix and Occultest) and by the benzidine test (*2*). Urine sediments were examined microscopically for erythrocytes.

A definite hematuria ("4 plus") occurred in all of the experimental rats given 20 percent and, later, 40 percent ethanol in lieu of drinking water. With practically no exceptions, the hematuria persisted throughout the experimental period of 58 weeks at the end of which the rats were killed. With rare transient exceptions in older animals, the control rats, which had been given water as the sole drinking fluid, did not show hematuria. Erythocytes were observed in the urinary sediments of a number of the treated group.

With respect to the amounts of hemoglobin in the blood as determined by alkalihematin (*3*) and cyanmethemoglobin (*4*) methods, the control and experimental rats were not significantly different. The control group had averages of 16.3 ± 0.9 and 16.1 ± 1.2 g of hemoglobin per 100 ml of blood as determined by the two methods, respectively, whereas the treated group had averages of 15.9 ± 0.9 and 15.9 ± 0.8 g/100 ml, respectively. This