Technical Papers

Dissociation of Chick Embryo Cells by Means of a Chelating Compound

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A number of recent investigations have involved the dissociation of the component cells of an embryo or organ rudiment (*1–5*). The dissociative agents have been high *p*H (*1, 4*), tryptic action (*2, 5*), or mechanical grinding (*3*). In two current problems, we found that the chelating compound di-sodium ethylene diamine tetraacetic acid—di-sodium versenate (*6*)—is very effective for dissociating cells from chick embryos (*7*).

One of these problems involves the dissociation of entire definitive primitive-streak-stage chick embryos into a suspension of cells. This is accomplished on a fairly large scale by the following procedure: The blastoderms are removed from the yolk, the area pellucida is dissected free in Tyrode's solution and then washed in three or four changes of Moscona's (*2*) Ca- and Mg-free solution. The blastoderms (six to eight in number) are then pipetted into 15-ml centrifuge tubes containing 2 ml of the versenate solution (100 mg percent, made up in Moscona's solution) which has been brought to approximately *p*H 8.5 with 0.4 ml of 1-percent KOH. After 20 to 30 min incubation at 37.5°C, the centrifuge tubes are agitated with a rotating motion. This agitation converts the blastoderms into a suspension of cells very rapidly. These cells may then be compacted by mild centrifugation, washed in Moscona's solution, and, again following centrifugation, resuspended in a standard tissue-culture medium (horse serum, Tyrode's solution, and embryo extract, in the proportions 2 : 2 : 1).

Microscopic examination prior to the last step reveals that the majority of cells are completely free and that the largest clumps contain only three or four cells. Very few of the cells show any visible indication of damage. Cells suspended by this procedure reaggregate in the culture medium. When such aggregates are kept in depression slides, they regularly form healthy cultures, which spread over the glass surface and live for at least 2 or 3 days. In a few instances, some of the larger aggregates have been placed on the chorioallantoic membranes of appropriate chick embryo hosts. They have yielded healthy, growing grafts.

In the second problem, we found it desirable to remove the ectoderm from the limb buds of 3-day chick embryos. The object was to leave the mesoderm as unaltered as possible. Trypsin skinning (*2*) was not appropriate for this work. It was found that the following procedure yields limb-bud mesenchyme which is firm and amenable to further manipulation: The limb buds are removed from the embryo surgically in Tyrode's solution, washed in three or four changes of Moscona's solution, and then placed in the versenate solution described in a foregoing paragraph (alkali not added). After 20 to 30 min at 37.5°C, the ectoderm is sufficiently macerated so that it can be scraped off (a longer sojourn in the versenate solution, especially at higher *p*H, results in the dissociation of the mesenchyme). This leaves the limb-bud mesenchyme smooth and firm and, after washing in Tyrode's solution or Tyrode's solution and horse serum (1:1), viable.

These observations suggest that di-sodium ethylene diamine tetraacetic acid may be a very useful adjuvant for other biological procedures that require Ca- and Mg-free solutions.

References and Notes

1. J. Holtfreter, *Symposia Soc. Exptl. Biol.* **2**, 17 (1948).
2. A. and H. Moscona, *J. Anat.* **86**, 287 (1952).
3. P. Weiss and G. Andres, *J. Exptl. Zool.* **121**, 449 (1952).
4. S. Mookerjee, *Nature* **171**, 796 (1953).
5. G. S. St. Amand and S. R. Tipton, *Science* **119**, 93 (1954).
6. Kindly supplied by the Bersworth Chemical Co., Framingham, Mass.
7. This investigation was supported in part by a research grant [C-1790 (R)] from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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Crystalline Neoretinene b

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We have prepared, in crystalline form, the isomer of vitamin-A aldehyde, which Hubbard and Wald (*1*) have called neoretinene b and identified as the precursor *in vivo* of the visual pigment rhodopsin. In this preliminary report (*2*) we describe the method of concentration and crystallization of the isomer, its physical properties, and its reduction to the corresponding vitamin-A isomer.

The preparative procedure was as follows: A solution of crystalline, all-trans vitamin-A aldehyde (4.0 g) in ethanol (1000 ml in a 1000-ml flask) was exposed for 4 hr to bright sunlight (outdoors, midsummer), filtered through a combination of Corning Nos. 3966 and 3850 filters. Alternatively, three photoflood lamps (No. 2), at a distance of 8 in. from the solution, were used as the light source, with the same filters, for an exposure time of 8 hr. These procedures proved to be preferable to the method used by Hubbard and Wald (*1*) for preparing larger amounts of the new isomer.

After removing the alcohol by distillation under reduced pressure, the residual mixed vitamin-A aldehyde isomers were dissolved in petroleum ether (bp 30° to 60°C) and chromatographed on a column (2 by 24