Reports and Letters

Human Amnion Cells for Large-Scale Production of Polio Virus

We wish to report a readily available source of large amounts of normal human cells that can be grown in mass tissue cultures and that support propagation of poliomyelitis virus. Large numbers of human placentae and membranes can be obtained in this country because of the many deliveries in clinics and hospitals. The high content of blood in the placentae and hyaluronic acid in the umbilical cord made it difficult to culture these tissues on a large scale. We have found, however, that the amnion and chorion lend themselves easily to washing, cutting, trypsin digestion, and growth in tissue culture.

The membranes from one delivery yield approximately the same quantity of cells as the kidneys of one monkey. Although Enders (*1*) has reported that cells emerging from fragments of amniotic membrane (2- or 3-mo gestation) are destroyed by poliomyelitis virus, this is not a suitable source of cells for routine culturing. We have found that cells of the full-term amnion support the growth of poliomyelitis virus and that the virus production is about the same as that in monkey kidney cells, which are currently being used to produce poliomyelitis virus for vaccine production.

In the present work (*2*) the two membranes were cut from the placenta and dropped into a phosphate-buffer solution at *p*H 7.2 containing penicillin and streptomycin. The amnion was stripped from the chorion, and each tissue was prepared separately. They were washed repeatedly in fresh changes of phosphate-buffer solution, and the chorion was gently scraped to remove the small blood vessels and clotted blood; this was not necessary for the amnion. The tissue was cut into pieces approximately 2 cm square and stirred gently in a 0.25 percent trypsin-buffer solution. The liberated cells were decanted at 20-min intervals, centrifuged at low speed, washed twice, and diluted with the culture medium. Plates, tubes, and flasks were seeded with cells from each tissue and with a mixture of cells from both. Although the membranes were usually worked up within 5 hr after delivery, it seems probable that they could be stored for longer periods of time without injury to the cells.

The cells of primary and secondary cultures grew equally well in media containing homologous or heterologous serums: cord, human, horse, ox, lamb. At a level of 20 percent, the serum yielded better growth than it did at a level of 40 percent. The medium for large-scale culturing consisted of 20-percent ox serum in either Parker's 199 or Earle's balanced salt solution containing 0.5 percent lactalbumin hydrolysate. Embryo extract gave no additional response and was omitted.

A cytologic preparation of the amniotic membrane, stained with Heidenhain's hematoxylin, showed a uniform layer of cuboidal, ectodermal cells. These were occasionally interlaced with much larger cells that stained less intensely. In tissue culture, the cells were uniformly epithelial, with two groups of cell sizes. The smaller cells were more numerous, and each flattened to cover an area of approximately 4 to 10 μ^2. The cytpolasm appeared homogeneous with few granules or vacuoles. The larger cells, which varied in number from preparation to preparation, each covered an area of 20 to 60 μ^2. They assumed bizarre forms and had prominent, parallel fibrous structures in their cytoplasm. The chorion consisted of a mixture of epithelial and fibroblastic cells. It yielded a heterogeneous population of cell types in tissue culture that was in contrast to the uniformly epithelial cells from the amnion. To date, successful preparations have been obtained from the membranes of 10 deliveries.

The amniotic cells were capable of infection with poliomyelitis virus, type I (Mahoney), type II (MEF-1), and type III (Saukett), and both the large and small cells underwent radical cytologic changes. The amount of virus produced by the cultures, measured as plaque-forming-units on monkey kidney plates (*3*), was the same order of magnitude as is usually obtained from cultures of monkey kidney cells. The susceptibility of chorionic cells to poliomyelitis virus infection is being determined, and further work is in progress on nutrition, cytology, the cytopathogenic effects of virus infection, and viral production in the cells from both membranes.

The results (*4*) indicate that human amniotic cells may provide a suitable alternative to monkey kidney cells for the large-scale production of poliomyelitis virus. The incidence of hepatitis or other extraneous viruses in such cell preparations must be determined, and adequate precautions must be taken to avoid complications caused by the possible presence of such viruses. The advantages of human, normal, nonorgan cells and their ready availability are obvious.

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References and Notes

1. J. F. Enders, *Ann. Rev. Microbiol.* **8**, 480 (1954).
2. This investigation was supported in part by a grant from the U.S. Public Health Service, National Institutes of Health, to P. L. Kirk and in part by grants to W. M. Stanley from the American Cancer Society, the Rockefeller Foundation, and Lederle Laboratories, Pearl River, N.Y. We wish to express our appreciation to A. W. Makepeace for providing the membranes and to Rosemary O. Lund for her technical assistance.
3. R. Dulbecco, *Proc. Natl. Acad. Sci. U.S.* **38**, 747 (1952); R. Dulbecco and M. Vogt, *J. Exptl. Med.* **99**, 167 (1954).
4. Edwin H. Lennette and H. H. Welsh of the Viral and Rickettsial Disease Laboratory of the California State Department of Public Health, to whom our preliminary results were communicated, have informed us that they have confirmed the ready growth of the amniotic cells and their infection with poliomyelitis virus.

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Extirpation of Roach Prothoracic Glands

In view of the recent observations on the extirpation of roach prothoracic glands by Chadwick (*1*), it is pertinent to report an almost identical, but shorter, series of experiments carried out in 1950–51 on *Blatta, Periplaneta,* and *Cryptocercus.* These operations, along with many others of an exploratory nature, were directed toward determining the role of the host-roach, *Cryptocercus punctulatus,* in producing the sexual cycles of its symbiotic flagellates (*2*). The cycles take place at the time of molting and have recently been correlated in chart form with the host molting period.

The technique employed here differed from that described by Chadwick only in the addition of a preliminary step that was designed to delimit these elusive glands. Each roach was given a small thoracic injection of 0.2-percent methylene blue in saline about 1 hr before the operation. The four ends of the X-shaped gland were then generally visible through the membranous ventral integument.

The results of these extirpations compare favorably with those of Chadwick and are summarized in Table 1. The total of nymphal roaches was made up of one *Blatta orientalis,* 13 *Cryptocercus,*

and 30 *Periplaneta americana*. A few additional animals that survived no longer than 4 days are omitted, since they never fully recovered from the operation. Of the 37 animals, 10 underwent two postoperative molts, and six underwent three such molts. Although only five individuals reached the adult stage in the first postoperative molt, 18 reached this stage at subsequent molts.

Twenty-one of the *Periplaneta* nymphs were operated on at varying times from a few minutes to 35 days after the molt. Those operated on more than 10 days postmolt had about 60-percent longer first postoperative instars. From this it might appear that the intermolt period had been significantly lengthened by prothoracic gland extirpation; however, it seems more reasonable to conclude that this postoperative instar extension resulted from the operation itself and the subsequent wound repair. The second and third postoperative instars of 16 and six roaches, respectively, were not unduly long. The intermolt periods of many domestic roaches, even among litter mates reared under controlled conditions, are highly variable (*3*). Because of this and the small number of individuals involved in these experiments, speculation on apparent delay in molting is not justified. The sexual cycles of the *Cryptocercus* protozoans were not affected by the extirpations.

In most cases, examination of the excised gland and subsequent autopsy showed a partial removal of 50 to 98 percent of the gland. In the cases in which 98 percent or more was removed, it could not be determined with absolute certainty by either of these methods whether a small part of one or more ends was left within the animal. Further, the gland frequently forms small branches along fine tracheae. The ease with which these were torn away left some question concerning the possibility of complete removal and consequent reliability of the operation. Although no significant regeneration was noted, it was felt that very small sections of the gland might be sufficient to enable an animal to continue its growth and development. At the time of these operations, and in the light of Bodenstein's findings (*4*), additional experimentation was deemed necessary before publication of these results. The need for further study of this gland, the brain, and perhaps even unsuspected hormone sources, is becoming increasingly apparent. This report is intended as an added stimulus to this end.

Table 1. Prothoracic gland extirpations: molting in the first postoperative instar

Extirpation (%)	Total	Died	Molted to adults	Molted to nymphs	% molting
98 or more	5	0	0	5	100
50 to 98	39	7	5	27	82

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References and Notes

1. L. E. Chadwick, *Science* 121, 435 (1955).
2. This work was supported in part by a National Science Foundation grant.
3. G. E. Gould and H. O. Deay, *Purdue Univ. Agr. Expt. Sta. Bull.* 451 (1940).
4. D. Bodenstein, *J. Exptl. Zool.* 123, 413 (1953).

6 April 1955

Doverite, a New Yttrium Mineral

Doverite, a new yttrium fluocarbonate, has been discovered at the Scrub Oaks iron mine at Dover, Morris County, New Jersey. The mineral is named for the city of Dover. It was discovered during the course of work being undertaken by the U.S. Geological Survey on behalf of the division of research of the U.S. Atomic Energy Commission.

The new mineral occurs in aggregates mixed with xenotime, hematite, and quartz. The aggregates are irregular—some of them are as large as 1 in. in diameter, and some of them have rims of bastnaesite.

In parts of the mine, doverite constitutes several percent of the gangue. It is anisotropic and has indices of refraction in the range from 1.700 to 1.685. No detailed optical data can be presented because of the finely crystalline nature of the mineral. The marked similarity of the x-ray diffraction powder patterns of doverite and synchisite ($CeFCO_3 \cdot CaCO_3$) indicates that the minerals are in the same crystal system and have the same crystal structure. The three strongest lines of doverite are 9.7, 3.53, and 2.78 A, which are almost identical with those of synchisite 9.7, 3.56, and 2.80 A.

Doverite is very fine grained and physically inseparable from the other components of the aggregates. Hematite and doverite were leached from the aggregates with concentrated hydrochloric acid; a residue of quartz and xenotime was left. Interpretation of chemical analyses of the aggregates shows doverite to be an yttrium analog of synchisite with the general formula $YFCO_3 \cdot CaCO_3$, the Y in the formula including several elements of the rare-earth group.

Doverite is brownish red and constitutes the bulk of the aggregates, which have a nonmetallic luster and a brownish streak, are brittle, and break with an uneven to subconchoidal fracture. Their hardness is 6.5, and the specific gravity is 3.89.

Chemical analysis of the aggregates shows the following percentages: rare-earth oxides, 44.36 (including Ce_2O_3 7.40); ThO_2, 1.62; SiO_2, 9.70; Fe_2O_3, 8.90; CaO, 9.80; P_2O_5, 8.75; Al_2O_3, 0.54; UO_3, 0.22; TiO_2, 0.75; MgO, 0.53; total H_2O, 1.35; CO_2, 11.75; and F, 2.87; total 101.14; less O = F 1.21; total, 99.93. Spectrographic analysis by K. E. Valentine of the Geological Survey shows Y to be a major component. The rare-earth components include minor amounts of Ca, La, Gd, and traces of Dy, Er, Yb, Nd, Pr, Lu, Ho, Tm, and Eu. Further detailed work on the minerals of this deposit is in progress.

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Orientation of Single-Crystal Silver Halides by Epitaxy

Attempts to orient single-crystal boules of AgCl and AgBr grown from the melt have been rather unsuccessful. These boules have the shape of cylinders and show no crystal faces. Orientation of the boules has been tried by (i) cleavage, (ii) punch figures, (iii) x-rays, and (iv) etch figures. Attempts to develop cleavage by striking a boule cooled in liquid nitrogen were not uniformly successful. These silver halides do not respond to the punch-figure technique of orientation because their glide elements $\langle 110 \rangle \{1\bar{1}0\}$ do not lead to prismatic slip. Our attempts to have the boules oriented by x-rays have not been successful, probably because of the high x-ray absorption of these salts. Further, the x-ray method does not readily give information on whether or not the boule is a single crystal. Etching with 10 percent $Na_2S_2O_3$ solution will reveal the grain boundaries in a boule consisting of more than one crystal but does not reveal the orientation.

Boules of AgCl and AgBr can be readily oriented by epitaxy of NaCl on the boule surface. This epitaxy (parallel oriented growth of NaCl on the silver halide) is produced by completely immersing the boule in a water solution of NaCl (saturated at room temperature) and allowing the solution to evaporate slowly in a constant-temperature room. After the solution has evaporated for several days, the boule acquires a coating of fine NaCl cubes (0.1 mm to 2.0 mm in size) in a close parallel-growth arrangement. The orientation of any portion of the boule can be readily seen from the integrated reflections from the (100) faces of these small cubes and can be accu-