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.

ELSA M. ZITCER
JØRGEN FOGH
THELMA H. DUNNEBACKE
Department of Biochemistry and
Virus Laboratory,
University of California, Berkeley

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.

3 June 1955

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,*