with respect to the isoimmune sera and what proportion of the molecules may have neither specificity. Also, it will be interesting to determine whether in such rabbits as B, the specificities of RGG-I and RGG-II are on the same or different molecules (*7*).

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22 October 1958

Development of a Chick Embryo Heart Cell for the Cultivation of Poliovirus

Abstract. An epithelial-like cell has been developed in line culture that apparently is stable. Although initially isolated cells were incapable of supporting the growth of poliovirus, the cells of the sixth and later passages allowed virus to propagate. The early, nonsusceptible cells were fibroblastic in appearance, in contrast to the epithelial type, poliovirus-susceptible, derived cell of later passages.

The use of cells in tissue culture for the propagation of poliovirus has been limited until recently to cells of primate origin. In 1957, Westwood, Macpherson, and Titmuss (*1*) developed a cell in line culture from embryo rabbit kidney. This cell was shown, by Sheffield and Churcher (*2*), to be capable of supporting the growth of the three types of poliovirus. Subsequently, Drew (*3*) and McCarthy and Tytell (*4*) reported the growth of poliovirus in other line cultures derived from rabbit kidney. Dunham and Ewing (*5*) demonstrated that cells derived from the chorioallantoic membrane of chick embryos were susceptible to the three types of poliovirus after five serial passages. Although it seems clear that cultures other than those of primate origin are capable of acquiring susceptibility to poliovirus when carried in passage, there may be an inclination by some to explain the susceptibility to poliovirus on the basis of contamination of the nonprimate cultures with cells of other susceptible lines. The work described in this report was conducted under conditions designed to minimize the possibility of such contamination.

This study concerns the development of a cell line from trypsinized chick embryo hearts which is capable of supporting the growth of poliovirus. Particular care was taken to insure against the possibility of contamination by other cell lines at all steps of the procedure. Trypsinized preparations were made in an area not used for other line cell work. Particular care was taken to limit the possibility of bacterial contamination of the various cell passages. The cultures were examined for contamination by pleuropneumonia-like organisms, with negative results.

The hearts were removed from 15- to 19-day-old chick embryos and placed in 100 ml of Hanks' balanced salt solution in a 250-ml flask. Approximately 45 hearts were used in each batch. A magnetic bar was placed in the flask and agitated in a magnetic field for 20 minutes. The fluid was poured off and replaced with an equal amount of 0.25-percent trypsin (Difco 1–250). Agitation was resumed for 2 to 3 hours, at which time the fluid was poured off and centrifuged at 1000 rev/min for 5 minutes; the pellet was then resuspended in medium 199 (*6*) containing 10 percent calf serum and adjusted to *p*H 6.8. Cells were planted in roller tubes at a concentration of 600,000 per tube, as counted in a hemocytometer.

Figure 1 shows fibroblastic-like cells isolated in the first passage of culture, and Fig. 2 shows epithelial-like cells which appeared in the fifth passage of culture. As transfers progressed, the line cells throughout the entire sheet were consistently of this type. Usually each passage was incubated for 7 to 10 days. The first four passages had as their typical and predominant cell an elongated fibroblast-like structure. In each passage, however, a few epithelial-like cells were present, and these increased in number with each transfer until at the fifth passage the culture consisted of approximately half fibroblastic and half epithelial cells. At the sixth passage most of the cells were epithelial, and at the seventh, a complete epithelial sheet was seen.

The three antigenic types of poliovirus were placed on chick heart cells of the first and sixth passages. No replication of virus was obtained on the first transfer, but on the sixth transfer all three virus types were propagated, yielding titers of 5.6, 5.5, and 5.2 for types I, II, and III, respectively, as determined by a metabolic inhibition test (*7*) with monkey kidney tissue. Virus produced by these cells was identified specifically by the complement-fixation procedure described by Mayer *et al.* (*8*).

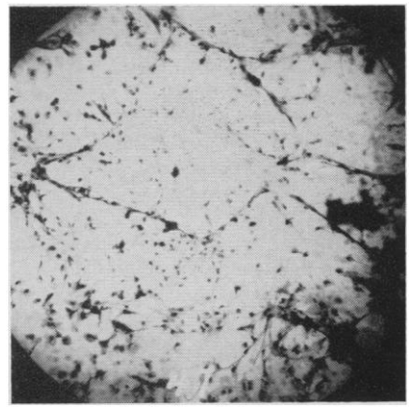

Fig. 1. Chick embryo heart cell, first passage (Giemsa stain). (About × 94)

It has been shown that several line cell cultures from various mammalian sources have a common antigen, as determined by complement-fixation techniques (*9, 10*). A. A. Tytell of the Merck Sharp and Dohme research laboratories (*11*) has examined the chick embryo heart cells and has found that those permitting poliovirus replication contained the common antigen, whereas the early passage strains resistant to poliovirus did not have the common antigen. Several explanations have been offered regarding this observation (*10*), and projects under consideration for the use of cells containing the common antigen for the production of virus vaccines must be deferred until the significance of the common antigen is understood.

It is of interest that a tissue culture series, originating with heart tissue, in which the cell type appears almost entirely fibroblastic has yielded an epithelial culture. The cell type has been obtained on two occasions from four attempts. One line now has been main-

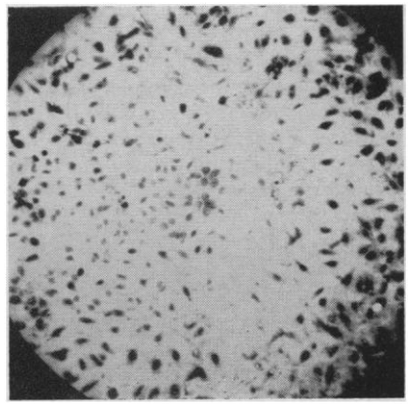

Fig. 2. Chick embryo heart cell, fifth passage (Giemsa stain). (About × 94)

tained for 15 passages and is stable for this period. There is little doubt, therefore, that in the present study the derived line cell originates from the primary tissue used (*12*).

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25 November 1958

Failure of Survival of Slowly Growing Members of a Population

Abstract. Water in which tadpoles or fish have grown inhibits growth of others of their own kind. Larger animals may completely suppress the growth of smaller ones and may eventually kill them by this water-borne inhibition. Under natural conditions of overproduction only the more rapidly growing would be expected to survive.

Work with various fish and tadpoles has indicated that each species as it grows releases growth-inhibiting products which act in feedback fashion. The inhibitory products, in the case of tadpoles, may be removed from the culture water by heating, freezing and thawing, centrifugation, filtration, or sonication (*1*).

The effect of larger animals on smaller ones is such that, for example, one *Rana pipiens* tadpole growing rapidly in 6 lit. of water with 3 lit. replaced daily will completely inhibit the growth of smaller *R. pipens* tadpoles.

Water from growing tadpoles inhibits the growth of smaller tadpoles. If food is withheld from large tadpoles their culture water is not inhibitory to smaller tadpoles. It seems that products of growth collect in the aqueous medium and tend to limit growth. The effect is more marked when the products come from larger tadpoles and are used on smaller ones.

Similar relationships have been observed with young, growing fish. A pair of White Cloud mountain fish, *Tanichthys albonubes,* produce many more fertile eggs in a 15-lit. aquarium than can grow to 1-cm size. No matter how many hatch, even as many as 200, never more than 20 reach 1-cm size. Shortly after feeding begins, differences in size appear. The larger fish continue to grow; the smaller ones stop eating and die in spite of an abundance of food.

There is nothing inherently wrong with the smaller fish. They can grow if they are removed to other aquaria, and all may live if the groups are smaller than 20. They can also grow in the original aquarium if their larger siblings are removed.

A more striking demonstration that products, rather than a deficiency of food, limit survival was obtained with another fish, *Barbus tetrazona.* This fish has larger eggs and can use as its first food small soil nematodes and granules of yolk from hard-boiled eggs. A slight excess of food was present at all times. From a spawning of over 200 never did more than 15 survive to 1-cm size in a 15-lit. aquarium. The survivors were always the most rapid early growers. The number of survivors to 1-cm size was increased to 174 by replacing one-half of the water two, three, and toward the end of the experiment, four times a day.

In view of the fact that the production of fish was increased more than tenfold by frequent water changes, it might seem strange that one large tadpole could completely inhibit smaller ones when water was changed frequently. This is not due to a difference between tadpoles and fish. The growth of a group of tadpoles all of the same size is also greatly increased by water changes. The important thing is that when larger and smaller animals are together, the inhibitory effect of the larger is so great that it is effective even when half of the water is replaced daily. This is true for both tadpoles and fish.

Under natural conditions of overproduction more organisms begin development than can survive. From the above results it is suspected that any genome which led to a decrease in growth rate would be a death warrant. A new genome that favored growth might spread rapidly, for its bearers would inhibit their more slowly growing relatives without being inhibited by them. This may be a relationship favoring rather rapid evolutionary advances in aquatic organisms (*2*).

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Neopilina (Vema) ewingi, a Second Living Species of the Paleozoic Class Monoplacophora

Abstract. In December 1958 the Lamont Geological Observatory research vessel "Vema" dredged four specimens of Monoplacophora from the Peru-Chile Trench off northern Peru. This is the second discovery of living representatives of this class of Mollusca which was thought, until 1957, to have become extinct in the Devonian. The specimens are considered to represent a new subgenus and species: *Neopilina (Vema) ewingi,* and the discovery suggests that more relict types may exist alive in the deep sea off Central and South America.

On 6 and 7 December 1958, members of the scientific staff aboard the research vessel "Vema" dredged four fresh monoplacophoran mollusks from two localities in the north end of the Peru-Chile Trench off Peru (stations 150 and 151). These specimens are considered to represent a new subgenus and species of the Cambrian-Devonian class Monoplacophora. As such they differ in several significant respects from *Neopilina (Neopilina) galatheae* Lemche, 1957 (*1, 2*), the other living species of this class trawled by the Danish ship "Galathea" off Costa Rica in 1952.

The localities at which the specimens were dredged are: station 150, lat. 7°35′S, long. 81°24′W, in 3183 to 3192 fathoms (corrected); and station 151, lat. 7°30′S, long. 81°25′W, in 3195 to 3201 fathoms (corrected). These localities are over 1300 miles south-southeast of, and 1200 fathoms deeper than, the Galathea station 716 (lat. 9°23′N., long. 89°32′W.) in 1963 fathoms (corrected) and are separated from that locality by the Cocos Rise.

Although analyses of ecological and geological data are still incomplete, in view of the wide interest in this class and its importance to paleoecology, molluscan evolution, and interphylum relationships (*3*), it seems advisable to publish this preliminary report (*4, 5*).

The specimens were collected by us, J. Lamar Worzel, chief scientist, Thomas G. Dow, of Lamont Geological Observatory, and Juan J. Rivero, a visiting

Table 1. Measurements of the types.

Length (mm)	Width (mm)	Height (mm)	Apex to anterior margin (mm)
Holotype, station 150			
15.5	14.0	5.0	3.0
Paratype, station 151			
12.5	10.7	4.5	2.0
9.2	7.6	2.9	1.5
Paratype, station 150			
4.9	3.7	1.5	0.8