metal chambers with windows capable of withstanding internal pressures up to 1000 atm. Such windows are necessarily quite small, and the possibility of mechanically manipulating the animals inside is very limited.

Since most of the animals of interest in this study (Brachiopoda and Pelecypoda) are filter feeders, a technique for detecting or measuring water flow would indicate the condition of the animal. Many techniques have been used (*1*) to study water transport in mollusks and brachiopods, but none are usable in high-pressure, limited-access environments.

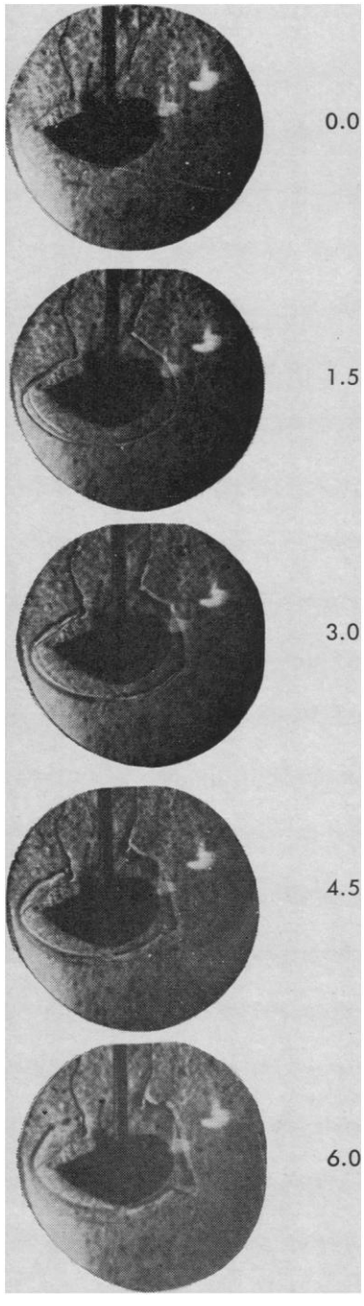

Fig. 1. The pumping action of *Mytilus edulis* at 1.5-second intervals (see text). The fixed, large, light-colored shapes are reflections from the window in the aquarium.

Schlieren optical techniques (*2*) were developed many years ago to detect changes in the refractive indexes of gases passing over high-velocity projectiles. We have applied these techniques to fluid-filled optical paths to detect small changes in index due to heating or variation in salinity; we can now look through a quite small window in a high-pressure aquarium and measure the water flow through and around a growing animal.

Any spatially nonuniform change of the refractive index in the light path in a Schlieren system will direct rays either behind the knife edge or completely free from the edge, causing the local area of the mirror behind the disturbance to appear either darker or lighter than the surroundings. A very small change in index creates a marked local brightening or darkening of the mirror: 0.1°C change in temperature or 0.5-per-mille change in salinity over a 1-mm path causes an easily visible change in brightness.

Figure 1 shows a small blue mussel, *Mytilus edulis*, supported on a plastic rod and encircled by a fine nickel wire that could be electrically heated to produce a small toroid of slightly heated water around the animal. The five photographs, in sequence at 1.5-second intervals, show successively the quiescent system (0), the heating water (1.5), distortion of the toroid by water exhausted from the mussel (3.0), further distortion (4.5), and convection of the whole toroid, plus two distortions due to exhaust from the animal (6.0).

Although the technique was developed for two-dimensional use, it is possible to use three orthogonal systems simultaneously to obtain a three-dimensional map of the water flow around an animal. It is also possible to drop individual grains of table salt around an animal to make a curtain of "threads" similar to the smoke streamers used in a wind tunnel; a single grain falling through the water produces a very fine opaque thread, which persists much longer than heated water. Details of the flow may also be studied by producing very small "blobs" of heated water locally by use of a very short heated wire.

Major advantages of this technique are absence of foreign material from the water circulated by the animal, least disturbance of the environment, suitability for use *in situ* or in very hostile environments, and vivid visual and photographic visibility in three dimensions.

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21 July 1965

Triploidy in a Human Cell Line

Abstract. *Cytogenetic studies of a human amnion cell line (strain RA) indicate that the major stem cell was triploid. Consistently present in the triploid cells were two marker chromosomes, a small centric fragment, and an apparently telocentric D. At various times triploid cells constituted 40 to 80 percent of the cell population. The possible origin of the triploid cell is a diploid/triploid mosaic amnion.*

Studies of chromosomal evolution of cells grown in vitro indicate that chromosomal change seems to follow a pattern of diploidy to tetraploidy to heteroploidy followed by emergence of stem cells and further selection among stem cell derivatives (*1*).

This report describes the karyotype of strain RA ("recovered amnion"), a continuous epithelioid line derived by spontaneous alteration from clinically normal, term, human amnion (*2*). Strain RA is of interest because its major stem line is essentially triploid (*3*).

Strain RA was used entirely for virological purposes during its early history and was not studied cytogenetically until the third year of culture. No data are available on the karyotype of the original culture.

In the present study, cells were grown on slides in wide-mouthed French-square bottles, on Eagle's medium (*4*) with 10 percent calf serum. After 48 hours of incubation, colchicine ($10^{-6}M$) was added to the medium for 6 to 16 hours. The medium was then removed, and a hypotonic Hanks solution (1 to 6 dilution) was introduced for 20 to 30 minutes. The cells were fixed in acetic acid–methanol (1 : 3),

Table 1. Chromosome numbers of 56 RA human amnion cells.

Number and percentage of cells	Chromosone number							Total
	65	67	68	69	70	71	72	
Number	1	2	1	1	45	3	3	56
Percentage	1.8	3.5	1.8	1.8	80.3	5.4	5.4	100

air-dried, and stained with Wright's stain.

Periodic enumeration of chromosomes of RA cells over a 3-year period indicated shifts in chromosome number that could be described by an interplay of one or several hyperdiploid stem cells with 55 to 61 chromosomes and an essentially triploid stem cell with 70 chromosomes. At various times triploid cells constituted 40 to 80 percent of the population. The forces which may bring about shifts in stem line predominance in cell cultures have been discussed in detail (*1*, *5*).

Table 1 presents the results of a detailed chromosome analysis of 56 RA cells. Triploid cells, at this time, constituted 80 percent of the population. These data were collected by a combination of careful counting and photographic karyotyping. Figure 1 shows the karyotype of a typical metaphase plate of the triploid fraction. The 70-chromosome cells appeared to be complete triploids with an additional small centric fragment. Triploidy is apparent with the acrocentrics of the D and G groups as well as with the A, B, and E groups. The remaining chromosomes fit the triploid number for the C group, assuming three X chromosomes, although the latter cannot be discerned. In all preparations, the short arm of one of the D group was not detectable. This chromosome was probably a telocentric. No typical Y chromosome was found, and there was no reason to believe the centric fragment was derived from a Y. The sex of the fetal source of RA is unknown. The centric fragment and the telo-D are excellent markers and have consistently occurred for 3 years in our cultures and in RA cells maintained in a separate laboratory by another worker (P. C. Loh).

With regard to possible origin of the marker chromosomes, measurements of the centric fragment suggest that it could be an isochromosome for the short arm of one of the D chromosomes. Therman *et al.* (*6*) discussed an apparent case of iso- and telo-D_L chromosomes derived in vivo. The case of Therman *et al.*, however, involved a mosaicism for the two types of cells, whereas RA cells presumably combined both types in one cell and would represent an isochromosome for a short arm.

Since it seems unlikely that the RA cell could arrive at triploidy by the circuitous route from diploidy to tetraploidy to triploidy assumed to be the case for most cell lines, it is possible that the original amnion was triploid or, more likely, a diploid-triploid mosaic. Several cases of triploid human embryos have been reported (*7*), but these did not survive to full term.

A continuous human cell line of triploid constitution offers unique opportunities for investigation of radioresistance, gene dosage effects, isozyme variations, and other genetic aspects of human cells in vitro, particularly when such studies can be done in parallel with the available diploid and aneuploid human cell cultures.

A clonal subline (RAC_1) was examined in the fourth passage, and tripoid cells constituted 90 percent of the population.

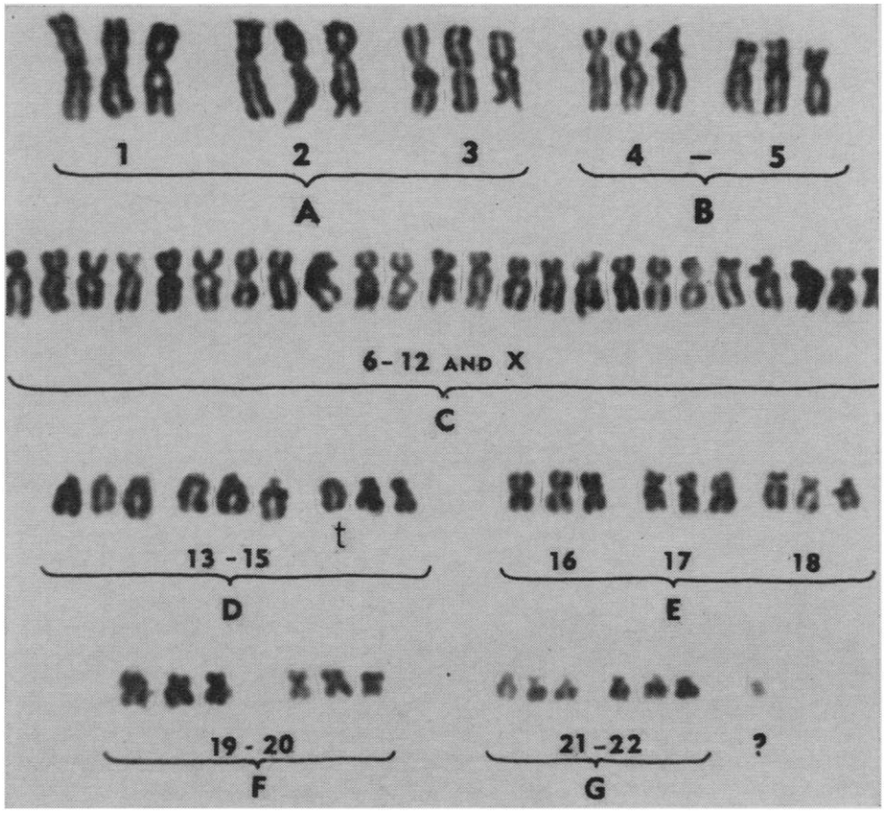

Fig. 1. Karyotype of a 70-chromosome RA cell. Note centric fragment (?) and telocentric (*t*) D chromosome.

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8. The technical assistance of Carmen Kanapi is gratefully acknowledged. This work was supported by a grant from the Hawaii Division of the American Cancer Society.
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