A Glass Helix Perfusion Chamber for Massive Growth of Cells *in vitro*. (27164)

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A number of technics for *in vitro* growth of animal cells have been developed. These may be classified into 2 general categories, namely cultures in which the medium is replaced at definite time intervals, and cultures in which a portion of the medium is continuously replaced by perfusion. Perfusion systems such as those developed by Graff and McCarty(1,2) seem to have an added advantage in that the cells are not alternately exposed to feeding and fasting periods. While this technic, which utilizes suspended cells, exhibits definite advantages, it cannot be used for all types of cells. For example, the Jensen sarcoma cells apparently require a solid surface to which the cells adhere before any extensive growth occurs. The present report describes a perfusion system which utilizes glass helices as a matrix for the cells.

Experimental. The cell chamber is a 1.2 × 75 cm ion exchange column which has been fused to the bottom of a 100 ml, standard taper, 3-neck, round bottom flask (Fig. 1). One side-neck is converted to a sampling orifice by removing the standard taper portion of the neck and inserting a 12 mm red serum stopper in this orifice. A 25 mm I.D. glass tube (6 cm long) is placed as a sleeve around the serum stopper, and the end is plugged with a bacterial gauze plug. This glass sleeve will permit the operator to sample the effluent medium as often as necessary without running the risk of chance contamination.

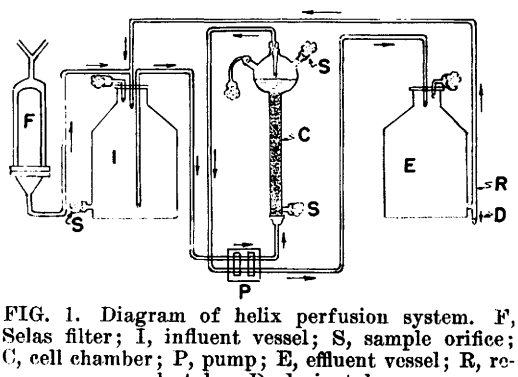

FIG. 1. Diagram of helix perfusion system. F, Selas filter; I, influent vessel; S, sample orifice; C, cell chamber; P, pump; E, effluent vessel; R, recycle tube; D, drain tube.

The effluent orifice is placed in the sample reservoir so the chamber and reservoir contain 40 ml of medium when the column is filled with helices. Another sample orifice is placed immediately below the fritted glass disc of the cell column which can be used for introduction of radioisotopes or any other test compounds.

The medium reservoirs are 5-gallon heat resistant polypropylene bottles with 6 mm orifices at the bottom. The 03 Selas filter is connected to the fresh medium reservoir by silicone tubing. All other connections are made with nylon tubing or pure gum rubber tubing. Before use, however, the rubber tubing is boiled in sodium hydroxide and rinsed thoroughly with glass doubled-distilled water. All glass connections are made with silicone high vacuum stopcock grease.

The perfusion pump is that developed by Graff and McCarty(1,2) with certain modifications. Fundamentally these include changes which would permit the pump to perfuse 12 cell chambers simultaneously and the use of a Master Gearmotor, Style 608042-FL* and a C-25 Motor Controller† to control the perfusion rate. This results in greater precision and reproducibility in the control of perfusion rates.

The complete assembly with 12 replicate cultures (Fig. 2) is autoclaved at 15 lb pressure for 1 hour and then evacuated for 40 min. The autoclaving procedure is repeated 48 hours later and the chambers are then placed in the incubator. Before the medium is sterilized, the fresh medium reservoir is gassed with a mixture of 5% CO_2, 20% O_2, and 75% N. Medium 5a(3) is passed

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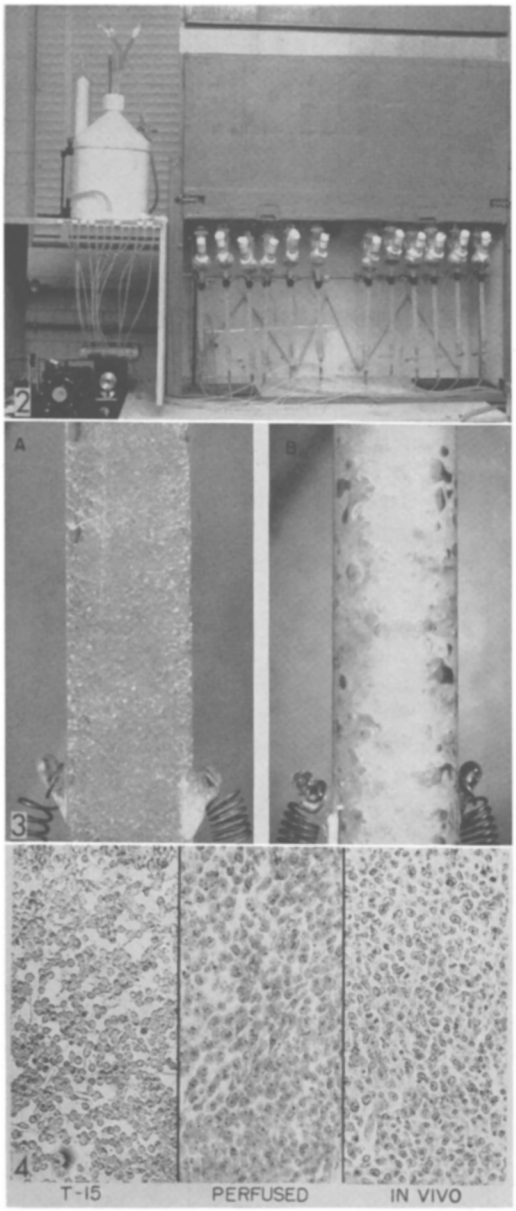

FIG. 2. Complete perfusion assembly with replicate cell chambers.

FIG. 3. Gross appearance of the helix perfusion chamber. A, 24-hr culture; B, 9-day culture.

FIG. 4. Comparison of growth patterns of Jensen sarcoma cells. ×32.

through the Selas filter under a pressure of 5 lb. Following this, the pump is charged and the medium is siphoned into the cell chambers. When the level of the medium passes through the fritted glass plate at the base of the column, the siphoning procedure is stopped. The freshly excised tumor cells were prepared as previously described(4,5), suspended in complete Medium 5a, and introduced into the cell column through the sample orifice. The cell chambers are left undisturbed for 3 hours, after which the perfusion is started. The initial perfusion rate is approximately 70 ml/24 hr, and any increases in this perfusion rate are determined by the pH and the glucose concentration of the spent medium. Whenever pH fell below 7.1 and/or glucose concentration below 170 mg %, the perfusion rate was increased. Growth rates could be followed by sacrificing individual cell chambers periodically, trypsinizing the cells, and making a viable cell count. Needless to say, a tandem of fresh medium reservoirs could be used in long-term experiments or individual reservoirs could be attached to individual cell chambers if desired.

In our studies, freshly excised Jensen, JA-1 and JA-2 tumors were used. The initial inoculum was 200,000 to 1,000,000 cells per ml depending upon the nature of the experiment, and the pH of the fresh, unspent medium was 7.4. When a bicarbonate buffer system was used, it was necessary to maintain a 5% CO_2 gas mixture above the fresh medium. As growth increased, it was necessary to supplement the bicarbonate buffer system with a 10 mM glycyl-glycine buffer.

Results and discussion. The gross appearance of the tissues in the cell chamber is shown in Fig. 3. Part A portrays the appearance of the helix column immediately after inoculation, and appearance of the cellular mass 9 days after inoculation can be seen in Part B. Note that the cell mass, which is clearly visible to the naked eye, tends to encircle each helix and form a solid, perfusable mass of tissue. In this particular experiment, an initial inoculum of 1,000,000 cells/ml was used, and 4 g (wet weight) of cells were harvested from one chamber at the end of 9 days. During the latter stages of the experiment, perfusion rate was increased to 4000 ml/24 hr and the cells utilized 2.5 g of glucose/day/chamber.

A comparison of Jensen cells as they appear under the microscope is shown in Fig. 4.

TABLE I. Growth and Reproducibility of Replicate Jensen Cultures in Helix Perfusion System.

Flask No.	Age of culture, days	Cell count ($n \times 10^6$)	Growth (inoculum = 1)	Effluent pH	Perfusion rate, ml/hr
4	3	5.8	.73	7.10	15
1	5	7.6	.95	7.10	18
6	8	23.3	2.91	7.05	20
2	9	37.2	4.65	7.20	36
3	9	36.9	4.61	7.10	36
5	9	26.7	3.33	7.35	36
7	9	36.2	4.52	7.10	36
9	9	35.1	4.38	7.10	36
10	9	37.8	4.72	7.10	36

The cells grown in the perfusion chamber appear to resemble tumor cells which were carried as an intramuscular transplant in Holtzman rats more than those cells in T-15 flasks. Furthermore, the mitotic activity of the perfused cells appears to be greater than those carried *in vivo*.

The presently reported perfusion system can recycle the medium for certain types of studies (Fig. 1). This can be accomplished by siphoning the spent medium into the fresh medium reservoir, adjusting the pH to 7.4 and replenishing the glucose concentration to 300 mg percent. When this was done the concentration of other constituents in the medium was sufficient to support growth for 2 recycling periods. The third recycle period, however, would not promote growth unless the medium was supplemented with glutamine, asparagine, and arginine in addition to the glucose. This observation merits further research.

Replicate cell chambers were then employed to determine the consistency of growth in the columns. The initial inoculum was 200,000 cells/ml and individual cell chambers were harvested at different time intervals. When the cells were definitely in the logarithmic growth phase, 6 chambers were harvested simultaneously and a viable cell count was made. From this it can be seen (Table I) that growth in 5 of the 6 chambers was remarkably consistent. The poor growth in Chamber 5 was anticipated, however, since the effluent pH was 7.35.

The use of helices as a cell matrix for tissue culture is not an original approach. Earle and co-workers(6) cultivated chick fibroblasts on glass helices and reported good growth. Their method of feeding, however, was more a periodic circulating system and not a continuous perfusion of fresh nutrients. It should be mentioned that the glass helices make cytological examination of the tissues somewhat difficult, but the use of nylon helices appears to circumvent this difficulty. Preliminary investigations in this area have been encouraging.

Summary. Freshly excised Jensen sarcoma cells have been cultivated in a helix perfusion chamber. Growth was luxuriant and masses of tissue were clearly visible to the naked eye. Microscopic examination revealed healthy cells with a high degree of mitotic activity. This system is readily applicable for massive growth of cells which cannot proliferate in free suspension.

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Received November 9, 1961. P.S.E.B.M., 1962, v109.