dium permitting satisfactory growth in monolayer cultures.

The medium as described for suspension culture appears to be nutritionally complete for the 6 cell lines tested. Growth was maintained continuously as cell populations of 5 $\times$ 10^5 to 1 $\times$ 10^6 cells per ml for several months by changing the medium on alternate days and reducing cell populations at the time of medium change. Maximal cell populations attained in the defined medium have approached those of the lactalbumin medium (5). Populations higher than those shown in the growth curves were obtained with L cells by increasing medium changes to twice daily, indicating a possible requirement for a better buffering system or addition of an adsorbant for toxic cellular products.

The defined medium is relatively simple in chemical composition and, combined with the suspension culture method, should be of value in studying aspects of cell physiology such as response to hormones, drugs, enzymes, etc., as well as in obtaining a basic knowledge of nutritional similarities and differences for various cell lines.

Summary. A chemically defined medium is described that supports the growth of L, HeLa, monkey kidney, rat spleen, guinea pig kidney, and cat kidney cells in continuous suspension culture. Cell populations of 2.0 $\times$ 10^6 per ml to 3.1 $\times$ 10^6 per ml were obtained. The complete medium contains 33 components plus antibiotics and phenol red. The requirement for each component was determined for growth of a cat kidney cell line.

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Received October 24, 1962. P.S.E.B.M., 1963, v112.

A Disposable, Constricted Tissue Culture Tube. (28039)

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The incubation of tissue culture tubes at a slight angle, whether in rolled or stationary tubes, has presented problems. Inclining culture tubes at an angle of about 5° was designed to localize cellular monolayers at the distal end of the tube. If held horizontal, the monolayers would be spread over a large surface, requiring correspondingly larger volumes of medium and extensive microscopic scanning for detection of cell morphology. Furthermore, the proximity of cells to the opening of the conventional tube, if unslanted, makes the culture more subject to contamination. Because of the slanted position, we have found it necessary to seed primary monkey kidney (MK) cells in tubes with 300,000 cells per tube, and other cells, such as the stable HeLa line, with 50,000 cells, to assure an adequate attachment of cell population. Only a small percentage of cells attach at the upper area of the distal third of the tube. The majority of the cells slide towards the butt of the tube, with increased attachment rates near the base. The largest portion of the inoculated cells attach at the butt and proliferate from this area towards the upper portion of the tube, eventually meeting the centers of cell proliferation in the upper areas. This produces a cellular monolayer but of varying texture. With growth, such cultures develop a tendency to slough from the glass at the base of the tube

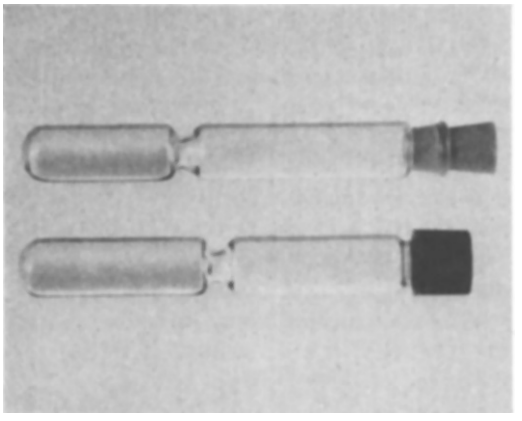

FIG. 1. Constricted tissue culture tubes 16 × 125 mm). The screw-cap tube is constricted 45 mm, and the rubber stoppered tube, 38 mm from butt.

due to an excess cellular population.

Leighton(1) designed a tube with a flat surface at the distal end for use with coverslips. The Leighton tube, which is incubated in the horizontal position, serves the purpose for which it was designed, but it is not feasible for use for routine cultures. With the slightest degree of lateral tipping, cells in this tube slide to one side of the coverslip or to the lateral edge of the flat surface of the tube, and an irregular monolayer develops. Furthermore, the Leighton tube is too expensive for routine use of large quantities, such as required in viral titrations and neutralization tests.

This report is concerned with the description of a new culture tube,* inexpensive and thus disposable, which overcomes the difficulties described above. The new tube is made of soda-lime glass, and the inner wall of the working area is treated, under careful control, with a film of SO_2. This coating tends to neutralize and stabilize the inner wall and also serves as a protective barrier. This barrier minimizes changes in the glass surface from the time the tube is made until it is actually used.†

A standard culture tube (16 × 125 mm) was designed with a constriction (9 mm internal diameter) placed 31, 38, or 45 mm from the base of the tube (see Figure 1). For the 38 mm tube, the dam formed by this constriction is adequate to provide a reservoir that can retain at least 1.5 ml of fluid. In practice, 0.5 ml or 1.0 ml of medium containing cells is introduced into the tube, which is incubated in the horizontal position, so that the entire cell population is evenly distributed within the reservoir. The constriction does not interfere with inoculation of cell suspensions or with filling or decanting fluid from the tube. Although these tubes are made with constrictions at different locations, we have found that the 31 mm reservoir is adequate for observation of cell changes due to virus proliferation.

Cellular growth in the new tube is more rapid, and, of greater importance, more homogenous. Not only do cultures grow to a complete monolayer more rapidly, but fewer cells are required as inoculum. Furthermore, the monolayer is maintained for longer periods without cellular sloughing than in standard round tubes incubated at an angle. Table I shows a representative, comparative study performed with the new tube and with the conventional tube.

With the same suspension of MK cells, a seed of 300,000 cells per tube attained a monolayer in 4 days in the new tube as com-

TABLE I. Comparison of Growth Rates of MK Cells in Different Culture Tubes.

Cells seeded per culture*	Day on which a complete monolayer was attained		Longevity of cultures§ (in days)	
	Standard tube†	Constricted tube‡	Standard tube‖	Constricted tube‖
300,000	6th	4th	18	27
150,000	8th	5th	21	30
75,000	11th	7th	24	35

* All tubes received 0.5 ml of cell suspension in M-H growth medium (0.5% lactalbumin hydrolysate, 2% calf serum in Hanks' salt solution containing 0.2 mM $AlCl_3$).

† Incubated at a 5° angle.

‡ Incubated horizontally until monolayer formed.

§ Fluids were changed every 7 days to 1.0 ml of M-E maintenance medium (0.5% lactalbumin hydrolysate in Earle's salt solution). When cell sheet began to retract or to slough from glass, cultures were discarded.

‖ After monolayers had formed, all tubes were inclined at 5°.

* Available from Demuth Glass Works, Inc., Parkersburg, W. Va.

† The SO_2 film is readily removed with a single rinse in distilled water. Tubes treated accordingly have maintained MK cells as long as, and usually longer than, controls grown in borosilicate tubes.

pared to 6 days with the tubes from the same batch of glass without a constriction and incubated at a 5° angle. With 150,000 cells, a monolayer was formed in the new tube on the 5th day, but only by the 8th day in the standard tube. With 75,000 cells, the culture in the new tube was confluent on the 7th day, but in the standard tube 11 days were required.

Virus inoculation afforded no problem in these constricted tubes. The inoculum could be inserted past the dam, and dropped from the pipette directly over the cellular area, or it could be placed on the side of the tube and allowed to run down onto the cell sheet. Comparative titrations using both the new and standard tubes showed no differences in TCD_{50} when poliovirus was titrated using 0.1 ml inoculum per tube. Once a monolayer is formed and virus inoculated, the tube then can be incubated at the conventional slant. If the 31 mm reservoir tube is inclined at 5°, the entire cellular area is covered by 1.0 ml medium.

As the culture is uniformly distributed over the reservoir area, it is possible to use the culture for studying the effects of depth of medium with more accuracy than has hitherto been possible. By slanting the tube after inoculation of virus the cells in the butt end are subjected to less aerobic conditions than cells at the proximal end of the reservoir. In view of the extensive, uniform monolayer produced in these tubes, microplaque technics are more satisfactory than when carried out in standard tubes.

While the constricted tubes prevent the cultures from sloughing as they tend to do in conventional tubes, they do not limit the growth of adventitious agents in monkey kidney cultures. Foamy virus, B virus, and other agents often present in MK cells can be suppressed by using an aluminum ion-containing medium(2).

The lime glass tube described is inexpensive and can be discarded after a single use. An additional advantage of the disposable tube is one of safety to laboratory personnel (3). A serious hazard is introduced by reusing culture tubes. Since repeated flaming and sterilization of tubes produces structural changes and weakness in the glass, tubes used repeatedly often break in a spiral fracture during stoppering or during the tightening of screw caps, causing injury to workers. During the first year's use of disposable lime glass tubes for cultures, (about 5000 weekly) we have not had a single tube break during stoppering. However, during the previous 2 year period when rewashed and resterilized borosilicate glass tubes were used, 26 lacerations occurred in our laboratory from fracture of tubes under the strain of stoppering. A number of these were of a serious nature, requiring surgical attention.

Summary. The use of a newly designed, disposable tissue culture tube is described. The new tube contains a circular constriction forming a reservoir at the distal end of the tube. Thus cells may be incubated level rather than at an incline, as in the case of conventional tubes. Cells incubated level in these constricted tubes form a monolayer more rapidly than in standard tubes incubated at an angle, and fewer cells are needed as seed for cultures. Other advantages are the uniformity of the cellular monolayer in the entire culture and the prevention of cellular sloughing. A striking safety feature of the disposable tube is the virtual elimination of tube breakage during stoppering.

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Received October 25, 1962. P.S.E.B.M., 1963, v112.