TABLE I. Reproducibility of Cell Counts of 6 Replicate Suspension Cultures when Agitated by the Warburg Apparatus in Basal Medium with Horse Serum and Methylcellulose.

Day	1	2	3	4	5	6	7	8	9
Mean cell count $\times 10^5$	1.61	2.07	2.54	3.29	3.93	4.50	5.39	6.74	8.82
Standard deviation $\times 10^5$	2.73	7.29	6.81	10.18	14.96	15.00	17.02	20.33	52.79

vice in this laboratory produced growth curves superimposable on those shown here. After completion of the above studies, rapidly growing L cells from a spinner culture were obtained from Littlefield and propagated as described by him(2) using the Warburg apparatus in place of the spinner. Growth curves (Fig. 5) comparable to those shown by the cells in spinner culture were obtained with no intermittent lag period. Thus the smooth reciprocal motion of the Warburg apparatus can be used successfully for cultivation of suspension cultures.

A Warburg apparatus is a common item in many biological laboratories and suspension cultures can be grown by this method without additional elaborate or expensive equipment. The water bath offers greater precision of temperature control than is available with air incubators and all cultures are agitated at precisely the same speed and in the same manner, thus promoting good reproducibility of growth curves which is advantageous in many types of growth studies. Cells from the cultures can be subjected to Warburg manometric studies without greatly altering their environment. A refrigerated Warburg, such as was used in these studies, conveniently allows work with suspension cultures at low temperatures. The method could be combined easily with the continuous CO_2 detection method of Karler and Woodbury(8), which involves use of a Warburg apparatus, to measure the CO_2 output of suspension cultures at various stages of the growth curves.

Summary. A study was made of the growth of suspension cultures of L cells when agitated by a Warburg apparatus through use of specially designed holders for the culture flasks. The basal medium was supplemented with certain compounds as protectants for the cells. Logarithmic growth curves were obtained with serum-free and serum-containing cultures. Good reproducibility of growth curves of replicate cultures was obtained. Certain advantages for growing suspension cultures were realized.

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Plaque Production with Infectious Bovine Rhinotracheitis (IBR) Virus.* (28244)

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Infectious bovine rhinotracheitis (IBR) virus was isolated by Madin *et al.*(1) and shown to produce a cytopathic effect on various primary bovine embryo tissue culture cells. To our knowledge literature does not reveal plaque formation by IBR virus. This report

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TABLE I. Plaque Counts in Bovine Embryo Kidney Cell Monolayer Cultures Inoculated with Various Dilutions of IBR Virus.

Exp No.	No. plaques/plate when concentration of virus in inoculum was 10^{-5}	10^{-6}
1	41, 42, 37	6, 4, 4
2	35, 32, 39	4, 5, 3
3	40, 38, 41	4, 4, 5
4	48, 42, 46	6, 4, 4
5	39, 42, 44	5, 4, 5

describes the production of plaques by IBR virus on primary bovine embryo kidney cells.

Materials and methods. Bovine embryo kidney cells. Cell cultures of primary bovine embryo kidney were prepared by using trypsinization technic of Youngner(2). Cells were suspended in Medium 199 containing 10% calf serum; 8 ml quantities were delivered to 60 mm diameter Petri dishes. Plates were kept at 36° to 38°C in humidified atmosphere of an incubator continuously flushed with a mixture composed of 97% air and 3% CO_2. Sheets developed in 5-7 days. *Virus.* IBR virus was received as a tissue culture supernate from Lederle Laboratories. Stocks were prepared by propagating the virus in primary bovine embryo kidney cells, then freezing and thawing. The fluid used as inoculum contained about 10^7 plaque forming units (PFU)/ml. IBR bovine hyperimmune serum was also supplied by Lederle.

Results. Plaque formation by IBR virus on bovine embryo kidney cells. The plaque forming ability of IBR virus was tested by using Dulbecco's(3) agar-overlay technic in which monolayers were prepared and inoculated with appropriate virus dilutions and incubated at 37°C for 1 hour. Each plate then received 6 ml of high-cystine altered Eagle's maintenance medium ($hcAE_m$)(4) containing 0.9% agar which had been washed by the procedure of Dulbecco and Vogt(5). Following 7 days incubation in the continuously flushed incubator a second agar-overlay containing 0.01% neutral red was added. After 2-3 hours clear plaques 5-7 mm in diameter were visible. The number of plaques produced was directly proportional to the concentration of virus in the inoculum (Table I), and the number of PFU was 1×10^7/ml. *Comparison of IBR plaque titers by Dulbecco's and Postlethwaite's methods.* The plaque titer of IBR virus was measured and compared by using Dulbecco's agar-overlay and Postlethwaite's(6) liquid overlay methods. In both methods primary bovine embryo kidney monolayers were washed twice, each time with 4 ml of phosphate buffered saline (PBS) and inoculated with 0.3 ml/plate of appropriate virus dilutions; 1 hour adsorption at 37°C was allowed. One-half the plates received 6 ml of $hcAE_m$ containing 0.9% washed agar (this procedure followed Dulbecco's method); the second half of the plates received 6 ml of $hcAE_m$ without agar in accordance with Postlethwaite's liquid overlay method. All plates were incubated in a continuously flushed incubator at 36°-37°C for 7 days. The agar-overlay plates were stained with 0.01% neutral red and scored. The liquid medium was removed from the other plates which were then stained for 10 seconds with strong carbol fuchsin, washed with water and dried in air. Plaques obtained when Postlethwaite's method was used were observed as unstained areas with a background of deeply stained red cells. The titers obtained with this method were slightly lower than those obtained when Dulbecco's method was used (Table II). *Identification of IBR plaques.* To verify that the plaques produced by IBR virus were not those of any other contaminating agent, the effect of IBR bovine antiserum on plaque formation by the virus was tested. Tube neutralization and plaque blocking tests were employed. In the tube neutralization method equal parts of IBR virus and antiserum diluted 1:5 were mixed to give 100 PFU/0.3 ml and incubated 1 hour at 37°C. Plates were inoculated with 0.3 ml

TABLE II. Comparison of IBR Virus Titers by Plaque Methods of Dulbecco and Postlethwaite.

Exp No.	Plaque titers* Dulbecco's technic	Postlethwaite's technic
1	1.3×10^7	3.6×10^6
2	8.0×10^6	2.7×10^6
3	1.2×10^7	2.2×10^6

* Each titer was based on the counts of 5 plates; total number of plaques on the 5 plates varied from 17 to 195.

of this mixture and following 1 hour adsorption time were overlaid with $hcAE_m$. The same procedure was used in preparation of control plates except that PBS was substituted for the antiserum. In the plaque blocking method bovine embryo kidney cell cultures were infected with 100 PFU/0.3 ml of IBR virus and following 1 hour adsorption were overlaid with agar-$hcAE_m$ containing 2.5% antiserum. Control plates infected with IBR virus received maintenance medium containing 2.5% normal serum or 2.5% PBS. Plaque production was prevented by either method.

Summary and conclusions. Plaques were produced by IBR virus on bovine embryo kidney cells by both Dulbecco's agar-overlay and Postlethwaite's liquid overlay methods. The lower plaque titers obtained when the latter method was used were perhaps due to errors in scoring plaques which were not clearly distinct. The formation of plaques by IBR virus was blocked by IBR hyperimmune serum in both the standard tube neutralization test and by incorporation of antiserum into agar-overlay.

Plaquing of IBR virus on bovine embryo kidney cells provided a more quantitative means by which to study the host-range of this virus and a more precise system for the biological assay of infective IBR nucleic acids which may possibly be obtained from the virus.

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Spontaneous Variations in Pulmonary Venous Pressure in Intact Dogs.* (28245)

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It is well established that the systemic veins play an active role in regulation of the circulation. Previous studies(1,2,3) have shown that the pressure in the systemic veins fluctuates constantly, and these fluctuations may be of great magnitude. These spontaneous fluctuations in pressure reflect changes in volume and tone of the systemic veins. Rheoplethysmographic studies have demonstrated the existence of spontaneous alpha and beta volume deflections in the human digital circulation(4) and that the beta deflections reflect variations in postcapillary venous tone (5). Although there has been a relatively large number of studies on the systemic veins, studies on the pulmonary venous system have been few because of inaccessibility of the pulmonary veins in intact animals. However, such studies are important especially since as much blood flows through the cross-sectional area of the 4 pulmonary veins per unit time as traverses the cross-sectional area of the aorta. Thus, the pulmonary veins probably play an important role not only in control of the pulmonary circulation but also of the systemic circulation. Indeed there is evidence that the pulmonary blood volume and, therefore, the capacity of the pulmonary venous reservoir, influence cardiac output(6).

Recent development(7,8) and improvement

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