1000 μg groups would be 4.27 and 4.01 mg/100 g BW respectively. On this basis the DNA level in the 500 μg HCA group was 13% greater than controls ($P<.05$).

Summary. Effect of oxytocin, lactogen, and hydrocortisone acetate upon mammary gland involution was studied in rat. LGH retarded involution to a significant degree, by maintaining DNA 48% above control group. HCA at levels of 500 and 1000 μg retarded involution significantly when DNA was related to final BW. OXT by either subcutaneous or intraperitoneal administration was not effective in retarding involution. These hormones, however, appear to maintain some secretory activity of involuting mammary glands. Data also suggest OXT has little influence upon release of lactogen from anterior pituitary.

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Adaptation of Equine Abortion Virus to Earle's L Cells in Serum-Free Medium with Plaque Formation.* (27558)

CHARLES C. RANDALL AND LUCY A. LAWSON

Department of Microbiology, University of Mississippi School of Medicine, Jackson

One of us(1) has previously reported the adaptation of equine abortion virus (EAV) to HeLa cells grown in monolayers. Repeated attempts in the past to infect Earle's original or 929 clone cells with wild type virus from infected horse tissue or with hamster-adapted virus met with failure, and were abandoned until recently. The L cell strain adapted to serum-free medium by Merchant(2) has been used in this laboratory for metabolic studies. The usefulness of this system prompted repetition of efforts to adapt EAV. The following report deals with the successful attempt to adapt this agent to L cells utilizing virus which had been adapted to another tissue culture system (HeLa).

Materials and methods. Virus. The 109th passage of EAV in HeLa cells sonicated for 4 minutes was used as the original inoculum. Precautions were taken to centrifuge the inoculum for 10 minutes at 10,000 rpm to sediment thoroughly any cells that might have escaped the process of disruption. Passage material was routinely stored at −40°C until ready for use. After freezing and thawing 2-3 times and centrifugation to deposit the gross cellular debris, monolayer cultures were inoculated with 1 ml of undiluted material.

Tissue culture systems. The tissue culture cells used in this study were obtained from Dr. Donald J. Merchant, University of Michigan, and were designated L-M 929 which originally was a clone of Earle's 929 clone cells. The cells were routinely maintained in log phase suspensions, being agitated at 120 rpm in an Eberbach rotary shaker kept at 37°. Monolayers were obtained by seeding 4-oz Brockway glass bottles with 5 ml of cell suspension diluted with fresh medium to a concentration of approximately 2×10^5 cells per ml. The medium for all cultures was serum free and is referred to as YELP by Merchant(2). Excellent monolayers were obtained in 1-3 days.

Histological procedures. For histological studies 1 ml containing approximately 200,000 cells was introduced into Leighton

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tubes containing cover slips and incubated 2-3 days until monolayers were formed. Suitable virus-inoculated cultures and controls were fixed in neutral buffered formalin and stained with hemotoxylin and eosin at various time intervals.

Complement-fixation methods (CF). The conventional method used in this study has been employed elsewhere for identification of the EAV which was adapted to HeLa cells (1). The origin and preparation of the various positive and negative control sera and antigens and the pertinent technics were the same as those reported previously. For preparation of tissue culture antigen, L cells were held at –40°C until ready for use. Preparations from a given passage were pooled, centrifuged to sediment the cells, and the sediment was then resuspended in a small portion of the supernatant and sonicated in a 9 KC sonic oscillator for 5 minutes. The tissue culture antigen was clarified by centrifugation at 2,500 rpm for 10 minutes. In the test proper, 0.25 ml of a 1:10 dilution of horse antiserum was pipetted into tubes containing 2-fold 0.25 ml of serial dilutions of antigen and 2 full units of guinea pig complement in 0.5 ml. Following overnight fixation at 4°C, 0.5 ml of 3% suspension of sensitized sheep red cells was added, and the tubes incubated for one-half hour at 37°C.

Virus titration. An estimation of virus infectivity was determined by the tube method ($TCID_{50}$) using 0.5 log increments. Pilot assays showed day 4 to be a suitable termination period for reading the end point. The procedure for calculating infectivity was that of Reed and Muench as described by Parker (3).

Plaque method. Monolayers in rubber-stoppered 4-oz prescription bottles were inoculated with passages 36 and 38 of EAV adapted to L-M 929 and allowed to fix for 2 hours at 37°C. The monolayers were washed 3 times and YELP containing 1.5% was overlaid and allowed to solidify. The bottles were incubated in the inverted position for some days. Neutral red was not included, as preliminary experiments indicated that the number of plaques were diminished(4).

Results. Serial propagation. L-cell monolayers inoculated with 109th passage of HeLa cell-adapted virus evidenced some cytopathic reaction at the onset. With low magnification, at 24 hours there were numerous, single, dark, granular, rounded cells interspersed with clumps of dark-staining cells in which the cell boundaries could still be recognized. There were numerous cells, however, that remained spindle shaped and did not appear infected. At 72 hours most of the cells showed the changes described at 24 hours, and there was some sloughing into the supernatant. There was some irregularity in the amount of the apparent infection up to the fifth passage. Subsequently, the cytopathic effect appeared relatively uniform with beginning sloughing of cells at 36 to 48 hours, becoming pronounced at 72 hours. Characteristic, classical, intranuclear inclusions were first noted at approximately 12 hours, and by 24 hours practically every cell appeared involved. This lesion is characteristic of the infection, as previously described(1).

Titration of infectivity in L cells. The cytopathic effect of the virus may be used to measure the 50% end point, or $TCID_{50}$(3). Titers of 10^{-7} to 10^{-8} are readily reproducible.

Identification of tissue culture virus (CF). Table I indicates that virus grown in L cells has maintained its serological specificity which has not been altered in a sequence of hosts, passing from horse to hamster to HeLa cells, and lastly to L cells. The antigen titration end point is used here as the highest dilution of antigen which results in complete fixation of complement with a 1:10 dilution of known positive antiserum.

Plaques. Initial cytopathic effect is evident microscopically on the 2nd to 4th days, and small, gross plaque formation may be observed on the 4th day. Counts made daily

TABLE I. Complement-Fixation Reactions.

Antigens	Serial dilution of virus	Antigen titration end points
EAV antigen (positive)		20
L-M 929 cells (normal)		0
L-M 929 15th passage tissue culture virus	10^{-15}	10
L-M 929 29th passage	10^{-29}	10

show no increase in number from day 7 through day 21. Increase in size from 1 mm at day 7 to 3-4 mm occurs over the 21-day period. The last 4 days several bottles were incubated upright, and the plaques developed long necrotic streamers in the direction of gravity. The control cell cultures appear relatively normal during the entire period of incubation and are apparently viable as evidenced by the increase in plaque size. The only change noted in microscopic morphology is the increased granularity of the normal cells. The plaques microscopically (28× magnification) have a hyaline to necrotic appearance, and many have hollow centers.

Challenge of other host cells with tissue culture virus. Sonicated, undiluted preparations from the 10th and 25th passages were inoculated intraperitoneally into hamsters 3 weeks of age, and resulted in death in 3-6 days. Sections of the livers of these animals showed characteristic lesions(5). Materials diluted 10^{-2} sickened but did not kill animals. This is somewhat similar to the reactions obtained with HeLa passage virus(1). Dilution of L-passage virus diluted 10^{-5} regularly produced characteristic cytopathic effect in HeLa cell monolayers with typical intranuclear inclusions.

Discussion and summary. This report is an account of the adaptation of EAV to L-M 929 cells with identification by complement-fixation methods. Infected cultures show cytopathic effects which may be measured by both $TCID_{50}$ or plaque-counting methods. The failure of the virus to propagate in L cells (original) in early experiments is unexplained. During this period the cells were grown in horse and human sera which may have been inhibitory. The possibility that the L-M 929 strains could be directly infected by hamster-propagated EAV has not been excluded. The possibility of contamination of the L-cell strain used in this study with HeLa cells has been considered. The special chromosomal marker of the L-M 929 strain permits precise identification and has not changed, according to Hsu and Merchant(6). Furthermore, YELP has proven very toxic to the HeLa cell line, and cell growth is highly unlikely in this medium.

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Early Potentiation of the Vasoconstrictor Action of Norepinephrine by Guanethidine.* (27559)

FRANCOIS M. ABBOUD,† JOHN W. ECKSTEIN,‡ AND MICHAEL G. WENDLING
Hemodynamic Laboratory, Cardiovascular Research Laboratories, Department of Internal Medicine, State University of Iowa College of Medicine, Iowa City

The work of several authors(1-4) indicates that the pressor response to norepinephrine increases after intravenous administration of guanethidine. It is not clear from these observations whether the increased pressor response is caused by a greater cardiac output or by augmentation of the vasoconstrictor effect of the norepinephrine. The experiments to be reported here were done to determine the effect of norepinephrine on blood flow and vascular tone before and after intravenous

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† Advanced Research Fellow of Am. Heart Assn.

‡ Established Investigator of Am. Heart Assn.