havior of the cell in the various osmotic environments but determination of the actual leakage and the concentrations of the substrates before and after permeation would give more complete information.

Summary. The rapid osmotic dehydration of *S. marcescens* produced when an aqueous suspension of the organisms was rapidly and efficiently mixed with solutions of sodium chloride was followed from 2.0-3.0 milliseconds after mixing by the change in light scattering of the suspension. After a small decrease the light scattering rose to a maximum which depended on the concentration of sodium chloride. The increase in light scattering was associated with the shrinking of the bacteria during osmotic dehydration. When the bacteria were introduced into a solution of a permeating solute, urea, there was an initial increase in the light scattering followed by a decrease to a value almost equal to that produced by the suspension in water. These changes are probably due to the initial shrinking of the bacteria due to osmotic dehydration followed by swelling to the original volume as urea and water enter the cells.

I wish to acknowledge the technical assistance of Mr. John F. Blackmer and the advice and criticism of Dr. John B. Bateman.

1. Mitchell, P., in: Bacterial Anatomy, Symp. Soc. Gen. Microbiol., 1956, v6, 150.

2. Mitchell, P., Moyle, J., J. Gen. Microbiol., 1957, v16, 184.

3. Bovell, C. R., Packer, L., Helgerson, R., Biochim. Biophys. Acta, 1963, v75, 257.

4. Gibson, Q. H., Milnes, L., Biochem. J., 1964, v91, 161.

5. Conway, E. J., Downey, M., ibid., 1947, v47, 347.

6. Elo, J. E., Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo, 1937, v8, No. 6.

7. Hill, S. E., J. Gen. Physiol., 1929, v12, 863.

Received July 26, 1965. P.S.E.B.M., 1965, v120.

Comparative Effectiveness of Two Culture Techniques for Isolation of Rubella Virus.* (30592)

JOSEPH W. BURNETT AND CHARLES A. ALFORD, JR. (Introduced by T. H. Weller)
Department of Tropical Public Health, Harvard School of Public Health, Boston, Mass.

Initial techniques for isolation of rubella virus in cell culture were based upon demonstration of viral interference in primary cultures of African green monkey kidney (AGMK)(1) and recognition of cytopathogenic effects (CPE) as well as of viral interference in primary human amnion cells (PHA)(2). This paper reports a comparative study of the sensitivity of these two types of cell culture for the primary isolation of rubella virus. Additionally, isolations were simultaneously attempted by cultivation of human placental and fetal tissues, and by inoculating extracts prepared from portions of these tissues into cultures of PHA cells.

* Supported in part by a research grant (AI-01023) from Nat. Inst. of Allergy & Infect. Dis., Nat. Inst. Health, U.S.P.H.S., and by grants from Parke Davis and Co., United Cerebral Palsy Research and Educational Foundation, Inc.

Materials and methods. Twenty-four specimens collected from patients with clinical rubella were tested. These included 5 throat washings† and 19 products of conception. Specimens were processed as previously described(3). Products of conception were ground by hand (10-20% wt per volume of nutrient medium) and centrifuged; portions of the supernatant were used as inocula. Isolations were attempted either immediately after collection or after storage of materials at −55 to −65°C for periods of up to 8 years. Volumes of 0.2 ml were routinely sampled for virus and employed in the subculture procedures.

PHA cultures were prepared in this laboratory and maintained with bovine amniotic

† Certain specimens kindly supplied by Dr. Samuel D. Bell, Jr., Dept. of Microbiology, Harvard School of Public Health.

fluid medium (BAF)(2). AGMK cultures were purchased‡ and thereafter maintained with basal Eagle's medium with 2% chick sera(1) or with BAF medium when specifically indicated.

In one series of experiments, the sensitivity of the two systems was compared. Titrations of infectivity of the specimens were carried out as follows. The undiluted suspension and serial 10-fold dilutions thereof were inoculated into PHA and AGMK cultures using 4 to 5 cultures of each cell type for each dilution. Fluid material from each PHA culture was serially subinoculated to a fresh culture on 2 occasions at intervals of 6 weeks. At the end of each subculture, and at 4 weeks after final subculture, the PHA cells were challenged with approximately 1000 $TCID_{50}$ Sindbis virus. Fluids (0.2 ml) from each AGMK culture were transferred individually, twice at 10-day intervals. At the end of the first passage and after 10 days of the second passage, the AGMK cells were challenged with 100-1000 $TCID_{50}$ ECHO virus type 11. Following Sindbis or ECHO type 11 virus challenge, observations were made daily from the second to fifth day. Absence of CPE, characteristic of the challenge virus, in a culture at the time when complete degeneration had occurred in control cultures inoculated with challenge virus was interpreted to indicate presence of interference due to rubella virus. Titers were calculated by the method of Reed and Muench at the end of the original passage and of each subculture. These values are expressed as tissue culture interfering doses 50% ($TCIND_{50}$) per 0.2 ml of the original inoculum(1).

A slightly different technique was employed to compare the efficiency of PHA and AGMK cultures for the isolation of virus. Three cultures of each cell type were inoculated initially. Thereafter, subcultures were performed with pooled fluids. The AGMK method was as noted above, except that 3 passages were performed and that cells were maintained on BAF medium. PHA cultures were observed for rubella CPE. When CPE was detected the fluids from the entire group of PHA cultures were pooled and aliquots then added to fresh PHA cultures; presence of rubella virus was confirmed in PHA cultures by demonstration of interference after challenge with Sindbis virus. In the absence of rubella CPE, 2 subcultures were made at 30-day intervals; each was challenged approximately 30 days after inoculation.

Fragments of freshly collected conceptus were propagated either by the plasma clot technique or by introduction into an established PHA culture where they soon became attached to the cell sheet. Outgrowth from the fragments ensued, and in the latter instance frequently overgrew the PHA cells. Cultures prepared in this fashion were examined at intervals for CPE. After 21-50 days, fluid from the original cultures was transferred to 3 new PHA cultures. Thirty days later the passaged cultures were challenged with approximately 1000 $TCID_{50}$ of Sindbis virus.

The identity of the rubella isolates was confirmed as previously outlined(3).

Results. Table I summarizes simultaneous infectivity determinations of specimens in PHA and in AGMK cultures. Of 12 specimens demonstrated to contain virus, 9 were positive on primary passage in AGMK cells, whereas only 5 specimens interfered on primary passage in PHA cultures. Virus was demonstrated in an additional specimen after first subculture with use of each system, while 3 other specimens were positive after a second subculture in PHA cells. The amounts of virus in the specimens were not sufficient to permit comparative evaluation of the efficiency of the 2 systems to quantitate virus. However, with the exception of one specimen (B placenta—Table I), AGMK cultures detected smaller amounts of virus than PHA cultures. The subculture procedure detected minimal concentrations of virus, not revealed on primary passage in 2 of 12 instances with the AGMK technique and 5 of 11 instances with the PHA method.

Recovery of rubella strains after simultaneous inoculation of PHA and AGMK cultures is summarized in Table II. From 24 specimens, including those in Table I, 21 strains of rubella virus were recovered in both cul-

‡ Flow Laboratories, Rockville, Md. and Microbiological Associates, Bethesda, Md.

TABLE I. Results of Rubella Virus Infectivity Titrations of Clinical Materials Performed Concurrently in Two Types of Cell Culture.

		$TCID_{50}$/0.2 ml original inoculum according to cell type and passage level				
		African green monkey kidney cells		Primary human amnion cells		
Patient	Source of inoculum	Initial passage	First subculture	Initial passage	First subculture	Second subculture
T	Throat washing	10^0	10^0	Neg	Neg	Neg
N	" "	$10^{0.6}$	$10^{0.6}$	Neg	$<10^0$	$<10^0$
C	" "	$10^{1.5}$	$10^{1.0}$	$10^{0.5}$	$10^{0.2}$	$10^{0.2}$
Fe	" "	$10^{0.5}$	$10^{0.5}$	Neg *Neg	Neg Neg	$<10^0$ Neg
Fo	Placenta	$10^{0.9}$	$10^{1.1}$	$10^{0.2}$	$10^{0.4}$	$10^{0.4}$
N	Throat washing	$10^{0.7}$	$10^{0.7}$	$<10^0$ $10^{0.5}$	$<10^0$ $<10^0$	$<10^0$ $<10^0$
B	Placenta	Neg	$<10^0$	$10^{0.3}$	$10^{1.1}$	$10^{1.1}$
Lo	"	$10^{0.3}$	$10^{0.6}$	Neg	Neg	$10^{0.5}$
J	"	$10^{1.8}$	$10^{2.3}$	$10^{0.6}$	$10^{2.0}$	$10^{1.1}$
J	Fetus	10^0	10^0	Neg	Neg	$<10^0$
Lo	"	Neg	Neg	Neg	Neg	10^0
Li	Throat washing	$<10^0$	$10^{1.0}$			

* Repeat titrations of the same specimen.

ture systems, either on primary or subsequent passage. Additionally, each system detected 3 isolates from specimens negative by the alternative procedure. Data on the passage level at which strains were detected are indicated in Table II. Thirteen strains were recovered in PHA cells and 14 strains in AGMK cells on original passage. The first subculture yielded 4 additional strains in PHA cells and 6 strains in AGMK cells while the second subculture yielded 4 and one strain respectively.

To investigate different types of inocula, ground and centrifuged supernatants and cultured tissue fragments from 7 conceptal specimens were prepared and examined. Though virus was demonstrated in all 7 cultured tissue specimens, only 5 of the inoculated extracts yielded virus. In those instances wherein infected human fetal tissue fragments were layered onto PHA cultures, specific rubella CPE was demonstrable in the underlying amnion cells even though no CPE was observed in the cellular outgrowth from the individual fragments. Both the amnion cells and outgrowth of inoculated tissue fragments were protected from the destructive effect of superinfection with Sindbis virus. The cellular outgrowth from fetal brain, heart and skin-muscle as well as placenta resisted Sindbis challenge in the presence of rubella virus whereas the cell outgrowths from the rubella-negative fetal tissues did not.

Discussion. Recoveries from clinical materials were accomplished more rapidly in AGMK than in PHA cultures, although the systems appear equally sensitive if the requisite subcultures are performed. Further, the

TABLE II. Results of Attempts to Isolate Rubella Virus Simultaneously in PHA and AGMK Cultures.

Tissue culture system	Total No. specimens examined*	Total No. isolates	Passage level at which virus first detected		
			Original passage	1st Subculture	2nd Subculture
PHA	24	21	13	4	4
AGMK	24	21	14	6	1†

* Four throat washings and 20 specimens from products of conception.
† Second subcultures performed on 14 of the specimens.

demonstrated usefulness of repetitive subculture for detection of minimal quantities of virus has obvious practical implications. The advantages of each system may be combined for the isolation of rubella virus from clinical specimens. Both interference and CPE were demonstrable in PHA cells within 3 to 4 weeks after transfer of fluids from rubella infected AGMK cultures. We have encountered no agent other than rubella virus which produces interference in both systems and the unique rubella CPE in PHA cells.

It is generally accepted that 2 subcultures are sufficient for isolation of rubella virus when a AGMK culture system is employed. However, in one instance in this study, and rarely on other occasions, we have not demonstrated virus until a third blind passage had been performed in AGMK cultures.

Summary. AGMK and PHA tissue culture techniques are comparable systems for isolation of rubella virus from clinical specimens, although the AGMK system offers practical advantages. In order to demonstrate minimal amounts of rubella virus in either system multiple subcultures must be utilized. Employing Sindbis virus-interference, the agent of rubella may also be demonstrated in tissue explants in PHA cultures.

1. Parkman, P. D., Buescher, E. L., Artenstein, M. S., Proc. Soc. Exp. Biol. and Med., 1962, v111, 225.

2. Weller, T. H., Neva, F. A., ibid., 1962, v111, 215.

3. Alford, C. A., Neva, F. A., Weller, T. H., New Eng. J. Med., 1964, v271, 1275.

Received July 26, 1965. P.S.E.B.M., 1965, v120.

Influence of Miotics, Diamox and Vision Occluders on Light-Induced Buphthalmos in Domestic Fowl.* (30593)

JEAN K. LAUBER, JAMES MCGINNIS AND JAMES BOYD
Department of Animal Sciences, Washington State University, Pullman

Enlargement of the avian eye by continuous light has been reported(1). Several of our recent papers deal with attempts to characterize and explore further the nature of this light-induced buphthalmos(2).

This eye condition in domestic fowl resembles human glaucoma in many ways, yet it can be brought about by a relatively simple adjustment of the photoperiod. It was of interest to investigate the response of affected birds to several of the drugs used for glaucoma therapy. In addition, several types of vision occluders were used unilaterally, in order to test whether continuous light affects the eye by local or systemic route.

In Experiment 1, male broiler type chicks (Delaware × New Hampshire cross) were assigned on the day of hatching, 5 birds per group, to one of several treatments: 1. Control. Under a diurnal photoperiod of 14 hours light per day (14L/10D). 2. Experimental. Under continuous light (24L/0D), in the following groups: (a) Untreated. (b) Right eyelids sutured shut with nylon thread. The nictitating membrane was not sutured. (c) An "eye patch" affixed over the right eye to exclude light without applying pressure to the eye. This patch was made from a triangle, approximately 2 cm on a side, cut from a black-painted ping pong ball. The patch was lined with a thin layer of foam polyurethane and affixed to the skin by sutures at the 3 corners (Fig. 1). (d) Diamox acetazolamide (Lederle) given orally, 50 mg/100 g feed (*ca* 7 mg/chick/day). (e) Diamox, 500 mg/100 g feed (*ca* 70 mg/chick/day). (f) Floropryl (DFP), (Merck, Sharp & Dohme), 0.1% isoflurophate in anhydrous peanut oil, several drops in the right eye daily for 6 weeks. (g) Humersol (Demecarium bromide, Merck, Sharp & Dohme), several drops in the right eye daily. The stock solution, 0.25% aqueous, proved to be fatal

* Scientific Paper No. 2705. College of Agriculture, Pullman. Project 1677. This research was supported in part by grant NB-03770 from U.S.P.H.S.