clear if one observes that the z-coordinate on a minimal surface is a harmonic function on the surface, so that one may complete it to an analytic function $z + i\zeta$, and apply the above methods.

THE PROPAGATION OF MULTIPLE VIRUSES IN CHICK KIDNEY CULTURES*

BY HUNEIN F. MAASSAB

DEPARTMENT OF EPIDEMIOLOGY, SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF MICHIGAN

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Introduction.—Efforts have been directed toward developing another tissue culture system in which viruses like influenza, adenovirus, measles, hemadsorption type I and II, and poliovirus can be propagated. The ability to grow all of these viruses in the same type of cells would permit a comparison of many of their biologic properties. Buthala and Mathews[1] have investigated the susceptibility of cultures of embryonic chick kidney cells to different animal viruses and Wright and Sagik[2] followed up this work with the study of plaque formation by these viruses in the same culture system. We found that the kidney of a fully developed chick could be excised with greater ease than that of an embryonic chick, that the yield of cells was considerably higher, and that a number of laboratory passaged viruses could be propagated in the monkeys obtained. The system has also been found suitable for primary isolation of viruses.

TABLE 1

"VIRAL SPECTRUM" OF THE CHICK KIDNEY SYSTEM

Virus	Passage History†	Virus Titer* TCID$_{50}$ per ml. 0 days	3 days	6 days
Influenza				
A (A/AA/1/58)	TW-CK$_5$	1.5	4.5	5.3
B (Lee)	E$_4$F$_3$M48E$_{50}$-CK$_5$	2.0	3.8	4.5
C (JJ)	AE$_{43}$	1.8	0	0
Adenovirus	HeLa cells$_{20}$			
Type 4	CK$_5$	2.0	3.0	4.3
Hemadsorption				
Type I	MK-CK$_5$	1.8	4.5	5.3
Type II	MK-CK$_5$	2.3	4.7	5.0
Measles	Chick embryo cells$_{13}$-CK$_5$	1.3	2.8	4.0
Polio	MK-CK$_5$	1.0	2.7	3.8
Type I	Stool specimen-CK$_5$	1.3	3.3	4.3

* All titers are expressed as the negative log of the dilution which will infect 50 per cent of inoculated chick kidney cultures tubes.

† CK$_5$—The fifth passage in chick kidney cells; TW—throat washing; AE—Embryonate eggs (Amniotic route); E—Embyronate eggs (Allantoic route); M—Mouse; MK—Monkey kidney.

The data summarized in Table 1 show that the viruses studied grew readily in this type of culture. A line of virus which has been cultivated in a different tissue system requires a period of adaptation before it reaches maximal growth in chick cells, while a line directly isolated in chick cells multiplies to a relatively high titer promptly. The infectivity titers of some of the agents like influenza and polio were lower than expected when measured after five transfers. However, it can be

anticipated that with further passages and/or modification in cultural conditions, better titers may be obtained.

The advantages of the chick kidney system are: it avoids the use of continuous cell cultures; it is cheaper than monkey kidney cultures; the tissue is readily available in large amounts; and to date no "wild viruses" have been encountered.

Procedure.—In all instances, kidneys from 4 to 5 day old chicks were used. The cells were prepared by a modification of the procedure of Youngner.[3] The growth medium consisted of lactalbumin hydrolysate (90 per cent of 0.5 per cent solution in Hank's balanced salt) and 10 per cent calf serum. The suspension of cells obtained was diluted so that each ml contained approximately 3×10^5 cells. Various concentrations of cells were used to seed different sizes of specially washed glass culture containers. The growth medium was removed at the sixth day and a new medium composed of Eagle's basal solution[4] and 5 per cent calf serum was substituted. This procedure was found most suitable for obtaining a uniform monolayer of cells. The cultures were used at the eighth day. The maintenance medium used to propagate the different viruses consisted of double strength Eagle's solution and 2 per cent calf serum or, in the case of influenza virus, double strength Eagle's basal media without serum.

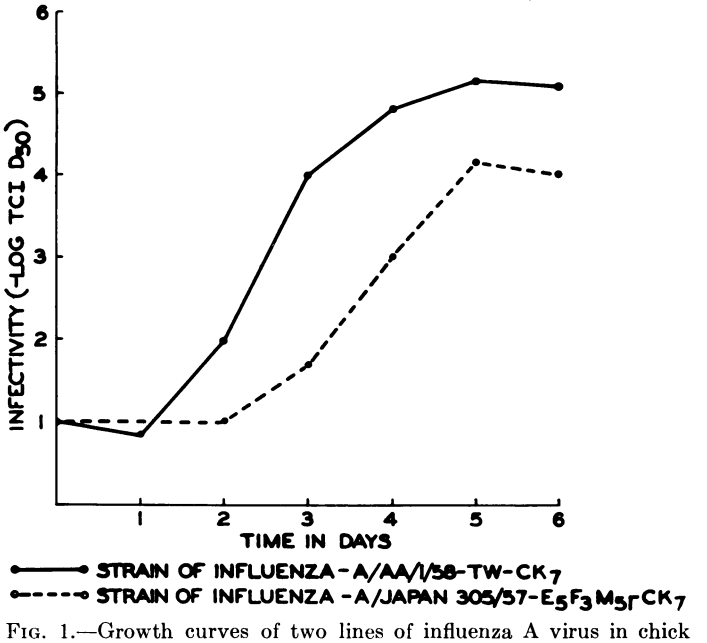

FIG. 1.—Growth curves of two lines of influenza A virus in chick kidney cultures.

Influenza Virus.—Both types A and B influenza virus produced cellular destruction during growth in the chick kidney cells. The infectivity titer of a line directly isolated from a throat washing was higher than titers of lines adapted to eggs or mice. The growth curves of two different lines of type A virus are shown in Figure 1. The data were obtained from cultures inoculated with about 50 $TCID_{50}$

of virus per cell. After three hours of incubation at 37°C, the cells were washed free of residual inoculum and fresh culture fluid was added. Samples were taken at daily intervals. It will be noted in the growth curve of the line isolated in tissue culture, that there is a lag period of about one day followed by a release period of at least three days. By the fourth day the maximal virus yield had been obtained since no further significant increase in titer was measured. Results obtained using the egg line of influenza A virus show a pattern of growth with a longer lag phase and the final virus yield was smaller.

Plaque Formation.—The plaque assay method of Dulbecco and Vogt[5] was modified for the use of chick kidney cell cultures. The cultures were prepared as described above. The cells were washed three times with Hank's balanced salt solution prior to the addition of virus. The strain of virus used was the Asian strain (A/AA/1/58) influenza A virus. The composition of the final agar overlay consisted of double strength Eagle's basal solution, 0.15 per cent Difco's Noble Agar, plus 100 units of penicillin, mycostatin, and 100 micrograms of streptomycin; finally solution was adjusted to pH 7.3 with 2.8 per cent $NaHCO_3$. The period of adsorption of the virus inoculum was four hours at 36°C before addition of the agar overlay. Each day, from the second to the eighth day following infection, neutral red (3×10^{-4}) was added and the number of plaques counted. It was found that influenza virus will produce plaques with an average size of 4 mm. The production of countable plaques started at the third day and progressed until the fifth day when the maximum number of plaques was reached. There was a nonspecific cellular degeneration after the sixth day of infection and it was found difficult to count the number of plaques with accuracy.

Measles Virus.—The Edmonston strain of measles virus will multiply in this culture system. Figure 2 shows a growth curve of the virus in chick kidney cells.

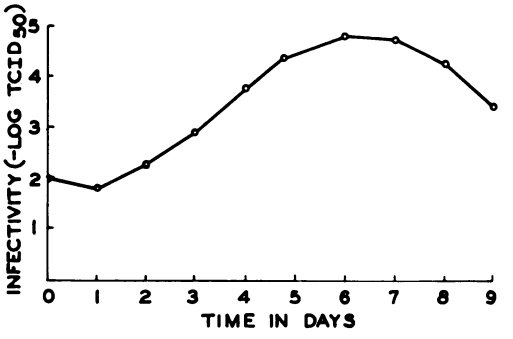

FIG. 2.—Growth curve of the Edmonston strain of measles virus in chick kidney cultures.

The virus did not require a period of adaptation. The maximum titer of about $10^{-4.3}$ is reached after approximately six days of incubation at 37°C. Cytopathogenic changes start at the third day and continue until the entire cell sheet is involved. The first increase in infectivity is evident on the second day of infection and progresses slowly until the sixth day. If the incubation of the infected culture is extended beyond the seventh day a marked decrease in infectivity titer occurs.

Adenovirus.—The chick kidney cells will support the growth of adenovirus, type 4. The rate of growth of the virus is slow. Maximum titer of $10^{-4.0}$ is reached on the seventh day. This is higher than ordinarily obtained in other systems. The progressive increase in titer from first to sixth passages is presented in Table 2.

TABLE 2

GROWTH PATTERN OF ADENOVIRUS TYPE 4 (RI-67*) ON SERIAL PASSAGES IN CHICK KIDNEY CULTURE

Passage Number	Infectivity Titer† ($TCD_{50/ml\ log}$)	Cytopathology
1	2.0	0
2	2.0	0
3	2.7	±
4	3.0	+
5	4.0	+
6	4.0	+

* The RI-67 virus was obtained from Dr. Hilleman and was passaged four times in HeLa cells in this laboratory.

† The values represent the reciprocal logarithm of dilution of the infectivity titer per ml at the 7th day after infection.

It will be noted that there was no increase in titer and there was no cytopathic effect during the first two passages. At the third passage a rise in titer followed by cytopathogenic changes was observed. The cytopathogenic changes were at first localized at the periphery of the monolayer culture. By the eleventh passage the changes occurred throughout the cell sheet. The amount of virus present was determined by disrupting the infected cells by quick freezing and thawing five times in an ethanol-dry ice mixture. Cultivation of other types of adenovirus has not yet been attempted.

Hemadsorption Viruses.—The growth pattern of the hemadsorption type I and II viruses is analogous to adenovirus type 4. Three serial passages in chick cells were required for adaptation of both agents as indicated by an increase in infectivity titer. The highest titer is reached on the sixth day after infection. Definite cellular alterations were evident on the third day and the hemadsorption reaction became positive approximately twelve hours prior to the appearance of cytopathogenic changes and the release of virus into the medium.

Poliovirus.—The Mahoney strain of poliovirus type I was found to multiply in chick cells. The infection of these cells by that virus is not efficient and the highest titer obtained at the eighth passage after five days of incubation at 37°C was $10^{3.7}$ $TCID_{50}/_{ml}$. The poliovirus persisted in the chick kidney cells with no concomitant increase for four passages. On the fifth passage an increase in infectivity titer and cytopathogenic changes occurred. The infectivity titer of poliovirus in chick kidney cells is lower than the corresponding titer in monkey kidney culture. The release of virus from the cells is extended over a 5-day period and the cytopathogenic effect is always confined to portions of the cell sheet. This is probably a reflection of the heterogeneity of the cell population with diverse cell types unable to support the growth of poliovirus. Attempts to obtain plaques with poliovirus in chick cells have been unsuccessful.

Isolation of type I poliovirus from stools was also successful. The infectivity titer and the rate of growth were higher and faster respectively for the isolate than for the adapted strain. The conditions for growth presented by the chick kidney cells are obviously different from those in monkey kidney cells; nevertheless, they were quite suitable for isolation of virus and for maintenance of poliovirus. The monkey cell strain was less well maintained.

Cytopathology.—In cultures similar to those in which the development of virus was followed, stained preparations of the cells were made at intervals. In this manner, it has been possible to identify infected cells, to describe the sequence of the cytological changes and to estimate the degree of involvement of the culture. Cultures infected with influenza virus show an increase in granularity, with rounding, enlargement of cells followed by pyknosis of the nucleus, ending in cellular fragmentation and death. The cellular changes as a result of infection with adenovirus type 4 are typified by rounding, and fusion of cells into clusters with increased granularity. The cytopathogenic changes associated with measles are similar to those described by Katz *et al.*[6] in chick embryo cultures. The involved cells exhibited a fusiform shape together with syncytia or multinuclear giant cells. Hemadsorption type I and II produce definite cytopathogenic changes characterized by focal areas of involvement with normal appearance of surrounding cells; the cytopathic effect of these two viruses are indistinguishable. The changes in cells infected by poliovirus consist of localized areas in which there is rounding, loss in cytoplasmic volume, increase in numbers of refractile cells, and pyknosis of the nucleus.

In summary, a chick kidney culture system has been developed in which a wide spectrum of viruses was propagated successfully. Factors have been described which influence the propagation of these agents. The growth of the viruses was always accompanied by cytopathic changes in the chick kidney cultures.

* This investigation was conducted under the auspices of the Commission on Influenza, Armed Forces Epidemiological Board, Office of the Surgeon General, U.S. Army, Washington, D.C.

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*THE RELATIVE HOMOGENEITY OF MICROBIAL DNA**

BY RONALD ROLFE† AND MATTHEW MESELSON

GATES AND CRELLIN LABORATORIES OF CHEMISTRY‡ AND NORMAN W. CHURCH LABORATORY OF CHEMICAL BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA

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Density-gradient centrifugation has revealed that the population of DNA molecules from *E. coli* is relatively homogeneous with respect to buoyant density in a solution of cesium chloride.[1] Because of the notably small apparent atomic volume of nitrogen in aqueous solutions of organic compounds[2] and because the guanine-cytosine base pair is more rich in nitrogen than is the adenine-thymine pair, it was considered that the density homogeneity among *E. coli* DNA molecules might reflect a high degree of homogeneity with respect to base composition. To investigate this possibility, an examination has been made of the relationship between buoyant density in cesium chloride solution and base composition of DNA from various sources.