CHICKENPOX AND HERPES ZOSTER
III. TISSUE CULTURE STUDIES

D. TAYLOR-ROBINSON*

From the Department of Bacteriology, University of Liverpool

Received for publication March 9, 1959

SERIAL propagation of virus from chickenpox or zoster cases has not been accomplished in laboratory animals, including the chick embryo, although the virus from zoster cases has been found to produce typical intranuclear inclusions in small pieces of human skin grafted on the chorio-allantois (Goodpasture and Anderson, 1944 ; Blank, Coriell and Scott, 1948 ; Caunt, personal communication).

In 1952, Weller and Stoddard reported the occurrence of eosinophilic intranuclear inclusions in cultures of tissue fragments of human embryo infected with chickenpox vesicle fluid. In a subsequent paper (Weller, 1953) it was reported that virus from several cases of chickenpox and herpes zoster had been propagated in roller tube cultures of human embryonic tissue ; but subculture was only successful when the inoculum contained infected cells. The cytological changes in the cultures inoculated with zoster vesicle fluid were indistinguishable from those produced by chickenpox vesicle fluid. Inhibition of the cytopathic effects of the virus in tissue culture was effected equally by convalescent zoster or chickenpox sera (Weller, 1958 ; Weller and Witton, 1958).

In this paper, experiments on the cultivation of viruses from chickenpox and zoster vesicle fluid in tissue cultures of human fibroblasts and of amnion cells are described ; and neutralization tests have been made which, like those of Weller, lend support to the view that viruses from chickenpox and zoster are immunologically identical.

MATERIALS AND METHODS

Human fibroblast cells

Fragment cultures in tubes were prepared by the method described by Feller *et al.* (1940). They were incubated on a roller drum. Human tissue, from 12–20 weeks-old embryos removed by hysterotomy, and human foreskin were used.

With embryonic fragments fibroblastic outgrowth was first seen on the 2nd day and by the 4th–5th day there was usually a luxuriant network of fibroblast cells. To maintain the cultures it was necessary to change the medium approximately every 3 to 5 days. With foreskin fragment cultures fibroblastic outgrowths appeared on the 3rd–5th day and the growth was not suitable for virus inoculation until the 10th–14th day. For these, it was necessary to change the medium about once every week.

Trypsin-dispersed cultures of human foreskin were prepared according to the method of Youngner (1954). Usually three foreskins were digested for 45–60 min. with 0·25 per cent " Difco trypsin 1:250 " made up in phosphate-buffered saline. The resulting cell suspension was removed and the extraction repeated with fresh trypsin 3 or 4 times. The cells in the pooled extracts were deposited by low speed centrifugation and resuspended in culture medium. The final cell suspension containing 5–10 $\times$ 10^5 cells per ml. was distributed in 1 ml. amounts to 6 $\times$ $\frac{5}{8}$ in. Pyrex rimless test tubes. A complete sheet of cells formed in

* Present Address : The Microbiological Research Establishment, Ministry of Supply, Porton, Wilts.

about 6 days. The medium was changed at least once a week. Cell suspensions for subculture were prepared by using 0·25 per cent trypsin ; in this way a " line " of human foreskin fibroblasts was maintained through 20 passages over a period of 26 weeks. All cultures prepared by trypsin dispersion of cells were kept stationary during incubation.

Human amnion cells

The successful growth of human amnion cells in a monolayer culture was first described by Zitcer *et al.* (1955) and their technique has usually been followed. The number of trypsin extractions of amnion was usually 5 or 6, the first of which was discarded. The final cell suspension was made up to contain 3–4 $\times$ 10^5 cells per ml. The cells were distributed in 1 ml. amounts to 6 $\times$ $\frac{5}{8}$ in. Pyrex test tubes and sometimes to flat-sided " medicine " bottles. For 6 oz., 4 oz. and 2 oz. bottles, 20, 14 and 5 ml. of cell suspension respectively were used. Each amnion provided sufficient cell suspension for 200–400 culture tubes. A change of medium on the 4th–6th day removed loose cells and accelerated the formation of a confluent cell sheet which was usually attained on the 7th–14th day. The medium was changed thereafter about once a week. Cultures sometimes began to degenerate after only 10–14 days, but in many instances cultures remained in good condition for 4 weeks or longer. Fibroblast-like cells sometimes appeared when the medium contained a high proportion of serum. This was especially liable to occur with a concentration of human serum greater than 20 per cent and was a reversible change.

Attempts to produce a " line " of amnion cells, as described by Fogh and Lund (1957), were not successful. Cells removed from bottles by trypsin never produced the confluent sheets in tubes shown by primary cultures. Hence subcultures of amnion cells were not employed.

Because focal lesions in amnion cell sheets tended to be more circumscribed and consequently easier to count than those in fibroblast cultures, amnion cells were used in most of the experiments recorded below.

Media

Initially, human fibroblast cells were grown in a medium containing human serum, for example :— 10 per cent inactivated human serum, 10 per cent inactivated horse serum, 5 per cent lactalbumen hydrolysate and 75 per cent Hanks' solution. When cultures were well established, the human serum was omitted from the medium.

An example of the type of medium in which human amnion cells were initially grown is as follows :— 10 per cent inactivated horse serum, 10 per cent tryptic digest broth, 5 per cent lactalbumen hydrolysate, 20 per cent bovine amniotic fluid and 55 per cent Hanks' solution. Sometimes the lactalbumen hydrolysate and the bovine amniotic fluid were omitted and other minor variations in the proportions of constitutents were made.

Inoculation of cultures

The inoculum consisted of vesicle fluid collected on the first few days of the eruption from chickenpox and zoster patients. The fluid was diluted immediately with 1·0 ml. of Hanks' solution or culture medium to prevent coagulation and, if not inoculated directly into tissue cultures, was stored at —70°. Before inoculation the medium was removed from tubes showing satisfactory cell growth and 0·1 ml. of diluted vesicle fluid added to each. After about 30 min. medium was added and the tubes incubated again at 37°.

Sera used in neutralization tests

Acute and convalescent sera from chickenpox and zoster cases were the same as those used in the complement-fixation and precipitation tests described previously. They were inactivated at 57° for 20 min. before use.

RESULTS

Cytopathic changes in cultures of human fibroblast and amnion cells

The nature and progress of lesions in chickenpox-infected cultures were never distinguishable from those in zoster-infected cultures, and the following description applies equally to both.

In sheets of fibroblast cells focal cytopathic changes were usually first observed after 4–6 days. These changes consisted in the formation of rounded, refractile cells. The lesions slowly increased in size by peripheral involvement of contiguous cells as described by Weller (1953) and Weller, Witton and Bell (1958).

In amnion cell sheets, again the first change, noted after 4 or 5 days, was a focal one. Cells became slightly more refractile but were not, at first, separated from their neighbours. By use of the collodion technique (Cheatham, 1954) sheets of cells were removed from the tubes and stained with haematoxylin and eosin. Infected cells contained eosinophilic intranuclear inclusion bodies as shown in Fig. 1. Multinucleated cells were frequently seen and in these each nucleus usually contained an inclusion (Fig. 2). Further incubation resulted in the " rounding up " and detachment of cells from one another. Again the lesions enlarged by the involvement of contiguous cells and the central cells became granular and necrotic. In a compact cell sheet the lesions were roughly circular (Fig. 3) rather than elongated as in fibroblast cultures. Eventually an amnion cell sheet would become completely infected and degenerate.

In one strain from chickenpox (CDT.) which has been passed repeatedly, a change in the character of cytopathic effects was noted in the 14th passage and has persisted in subsequent subcultures. In addition to the changes already described there were large syncitial masses with inclusion-containing nuclei gathered together in groups. This appearance was observed by Weller, Witton and Bell (1958), particularly in cells of epithelial origin.

The isolation and subculture of chickenpox and zoster virus strains in tissue culture

As observed by Weller (1953) the continued passage of virus strains in tissue culture was only successful if the inoculum contained infected tissue cells. At first cells were scraped off the walls of culture tubes which showed typical lesions and the resultant suspension of cells used as inoculum for fresh culture tubes. However, it was found that if cells from infected cultures were detached from the tubes and dispersed by the use of trypsin, the number of lesions produced on subculture could be greatly increased. The trypsinized cell suspension was centrifuged at 2,000 r.p.m. for 10 min. (625 *g*) and the deposit thoroughly dispersed in culture medium for inoculation into fresh cultures.

The strains of virus isolated and information about the time of appearance of lesions and subculture are shown in Table I. Virus was not grown from chickenpox vesicle fluid taken on the 4th day of the rash or later. From zoster cases vesicle fluid was occasionally infective as late as the 6th day from the onset of the rash. This difference may be due, in part, to the fact that zoster fluid was usually obtainable in greater amount so that cultures received a relatively more concentrated inoculum. There were no distinguishing morphological features among the 22 strains of chickenpox and the 21 strains of zoster. From one patient (ZWR.) virus was grown from the zoster vesicles and from the accompanying generalized varicelliform eruption. The earlier changes (increased refractility of cells) might be seen after 2 days, especially on subculture, although more often focal lesions were first detected after 4 to 6 days. Subculture was only attempted with some of the strains and these were usually only transferred through a few passages. One strain from chickenpox (CDT.) has been carried through 33 subcultures over a period of 22 months.

TABLE I.—*Strains of Chickenpox and Zoster Virus Isolated in Tissue Culture and their Continued Passage*

Chickenpox strains	Duration of rash in days when vesicle fluid taken	Days in culture before lesions first noted	Number of passages in tissue culture
CPL.	1	4	3
CLI.	1	4	0
CWI.	1	4	0
CZW.	1	5	0
CBU.	1	4	1
CIS.	1	5	1
CWO.	1	?	1
CAP.	1	6	0
CNI.	2	6	5
CPE.	2	7	0
CHO.	2	4	1
CFU.	2	4	1
CBL.	2	?	2
COB.	2	3	0
CDT.	2	5	33
CTH.	2	4	0
	4	—	0
CGS.	2	6	2
	3	6	1
	4	—	0
CDA.	3	4	1
CCO.	3	4	2
CPI.	3	9	5
CBI.	3	4	0
CUN.	3	6	1
	4	—	0
	5	—	0
Zoster strains			
ZTH.	1	4	1
ZTO.	2	2	0
ZTA.	1	4	2
ZBR.	2	4	1
ZHA.	2	3	1
	3	5	1
ZPE.	2	4	1
ZAB.	3	4	7
ZLO.	3	6	0
ZRO.	3	4	3
ZMA.	3	5	1
ZGU.	3	7	0
ZLA.	3	7	1
ZJO.	3	?	3
ZLY.	3	5	0
ZWH.	4	5	2
ZMO.	4	8	1
ZFO.	4	6	1
ZNE.	5	?	3
ZWR.	6	7	0
ZWA.	6	9 (2 lesions)	0
ZBO.	6	3	0

? = not recorded.
— = no focal lesions seen.
0 = strain of virus not subcultured.

The absence of virus from the fluid phase of tissue cultures

It was frequently observed in tissue cultures kept stationary that there was an increase in the number of lesions during the first 10 days. But if there were only 1 or 2 lesions at the end of this time, further lesions did not appear and progressive infection of the amnion sheet occurred through peripheral extension of the existing lesions. This suggested that there was no seeding of free virus to the healthy cells at a distance from the lesions.

On many occasions during the course of routine subculture, medium from infected culture tubes was removed, centrifuged at 2,000 r.p.m. (625 *g*) for 10 min. and the supernatant fluid inoculated onto fresh tissue cultures. Focal lesions never resulted. In one experiment, however, the medium from a bottle culture showing very gross cytopathic changes, was centrifuged at 2,000 r.p.m. for 10 min. (625 *g*). In 4 tissue culture tubes 5, 3, 5 and 4 lesions resulted from the inoculation of 1 ml. quantities of the supernatant fluid.

Weller (1958) reported that one virus strain of cytomegalic inclusion disease showed adaptation to tissue culture conditions, in that cell-free virus was demonstrated in the 3rd subculture. The chickenpox strain CDT. through 33 passages has not shown a similar adaptation nor has such been observed by Weller, Witton and Bell (1958) in their chickenpox-zoster strains.

Weller (1958) failed to release infective virus from tissue culture cells by various procedures designed to disrupt the cells. A similar failure has been encountered in these studies although a small amount of free infective virus has, on occasion, been obtained by using ultrasonic methods. These and other experiments concerning the close relationship between virus and tissue culture cells will be reported elsewhere.

The presence of free virus in vesicle fluid

Centrifugation experiments with zoster vesicle fluid suggested that extra-cellular virus was present in the fluid. A small sample (0·1 ml.) of vesicle fluid was diluted in 3 ml. of tissue culture medium and centrifuged at 3,000 r.p.m. (1400*g*) for 20 min. The top 1 ml. was carefully removed and added to a tissue culture tube. Similarly the next 1 ml. was examined and the deposit, resuspended in the last 1 ml. of fluid, was inoculated into a third culture tube. After 7 days the 3 culture tubes showed 70, 150 and several hundred lesions respectively. To demonstrate that this result was not due to the presence of cells or cell fragments in the supernatant fluid the following filtration experiment was carried out:—

Zoster vesicle fluid (0·3 ml.) taken from a patient on the 3rd day of the rash, was diluted with tissue culture medium to 4·0 ml. and 1·0 ml. was inoculated immediately into a culture of amnion cells. The remaining 3·0 ml. was filtered through a Gradocol filter of pore size 0·81 mμ. A positive pressure of 1–2 lbs./sq. in. was used and the filtrate divided between 3 amnion culture tubes, which were incubated at 37° for 6 days. The unfiltered fluid produced 80 focal lesions and the 3 tubes inoculated with filtrate showed 36, 18 and 14 lesions respectively. The filter was shown to be impervious to a culture of *Chromobacterium prodigiosum*. This experiment demonstrated unequivocally the presence of extra-cellular infective virus. Similar experiments were not performed with chickenpox vesicle fluid because of the lack of this material.

The effect of temperature on vesicle fluids and infected tissue culture cells

Vesicle fluids stored at $-70°$ have been found infective after 21 months, the longest period tested. The fall in infective titre in one zoster fluid stored at $-70°$ for 75 days was less than 50 per cent. On the other hand, in several experiments infected tissue culture cells were no longer infective after 24 hours at this temperature, although at 18° infectivity was maintained for several days. It would seem that the infectivity of chickenpox and zoster vesicle fluids stored at $-70°$ is dependent upon the existence of free virus therein. Virus within tissue culture cells, perhaps in an immature form, seems to be inactivated with the death of the cell, as was suggested by Weller *et al.* (1958).

Neutralization tests

In neutralization tests in tissue culture Weller and Witton (1958) used as their source of virus suspensions of infected tissue culture cells. They found that incubation of a suspension of infected cells with antibody-containing serum resulted in little inhibition of focal cytopathogenicity when the mixture was transferred to susceptible tissue cultures. However, the incorporation of the serum as an integral part of the medium of the cultures had an inhibitory effect. In the presence of convalescent serum there was a delay in the appearance of focal lesions and a significant reduction in their number. Chickenpox and zoster strains behaved similarly in neutralization tests in the presence of sera derived from either disease. In no instance was complete neutralization observed.

Tests with vesicle fluid as a source of virus

Because free virus had been demonstrated in zoster vesicle fluid it seemed that vesicle fluids would be of greater value than infected cell suspensions in which virus within the cell is presumably protected from the effects of immune serum.

The following technique was used initially:— approximately 0·5 ml. of zoster vesicle fluid (ZTA.) obtained on the first day of the rash, was mixed with 27·5 ml. of tissue culture medium (without horse serum). In $3 \times \frac{1}{2}$ in. tubes, 0·8 ml. quantities of the diluted vesicle fluid were then mixed with 0·2 ml. quantities of inactivated serum, acute or convalescent, from chickenpox or zoster patients. The tubes were shaken thoroughly and left at room temperature for $1\frac{1}{2}$ hr. The mixtures were then inoculated into tubes of amnion culture from which the medium

---

EXPLANATION OF PLATES

FIG. 1.—An early focal lesion of infected amnion cells 5 days after inoculation with zoster vesicle fluid. Note intranuclear inclusions. H. & E. × 290.

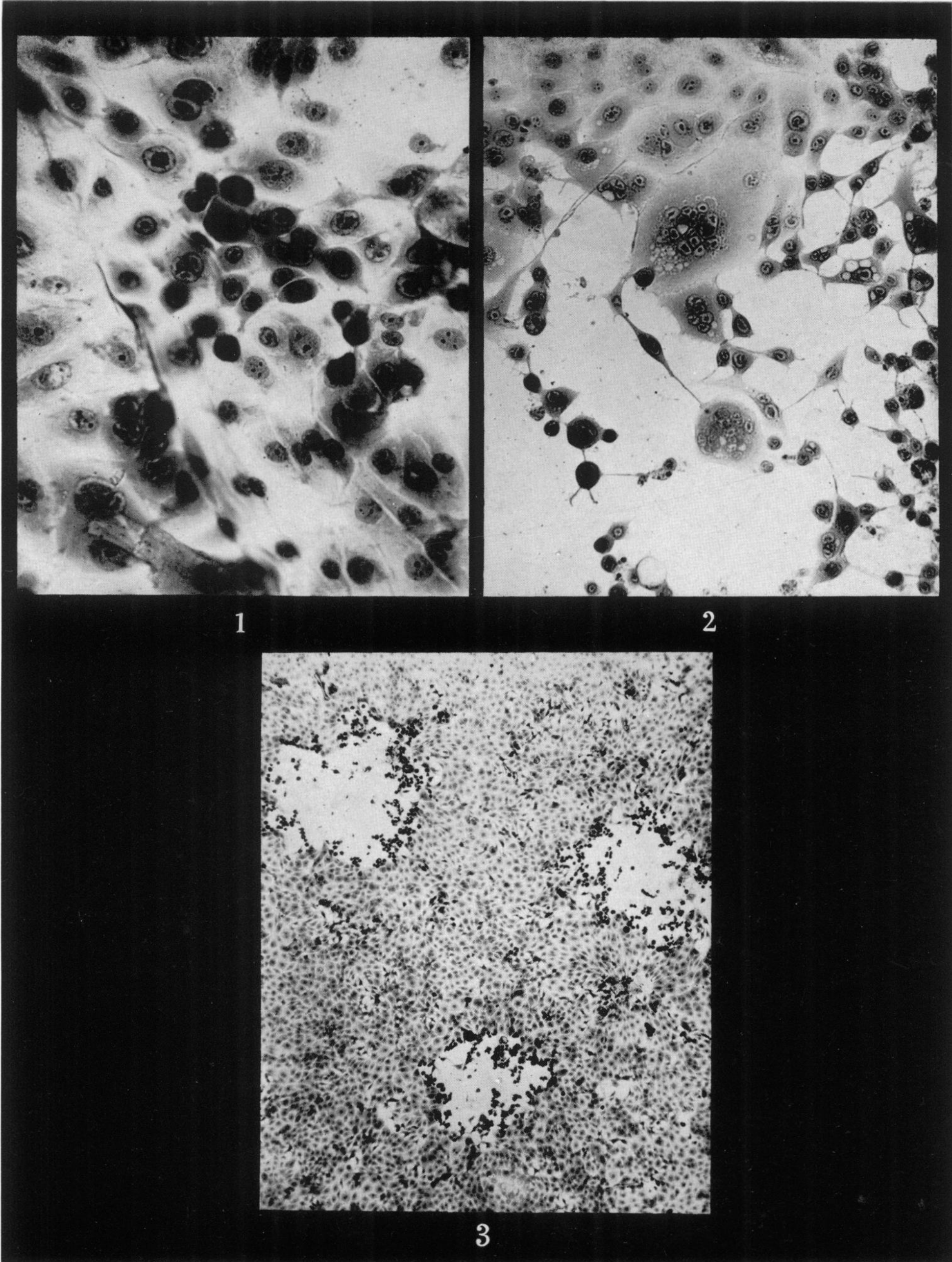
FIG. 2.—Typical multinucleated giant cells, each nucleus containing an inclusion body, in an amnion cell culture infected with chickenpox vesicle fluid. H. & E. × 240.

FIG. 3.—A compact sheet of amnion cells showing 3 focal lesions. The degenerate cells in the centre of the lesions have been lost in making the preparation. H. & E. × 29.

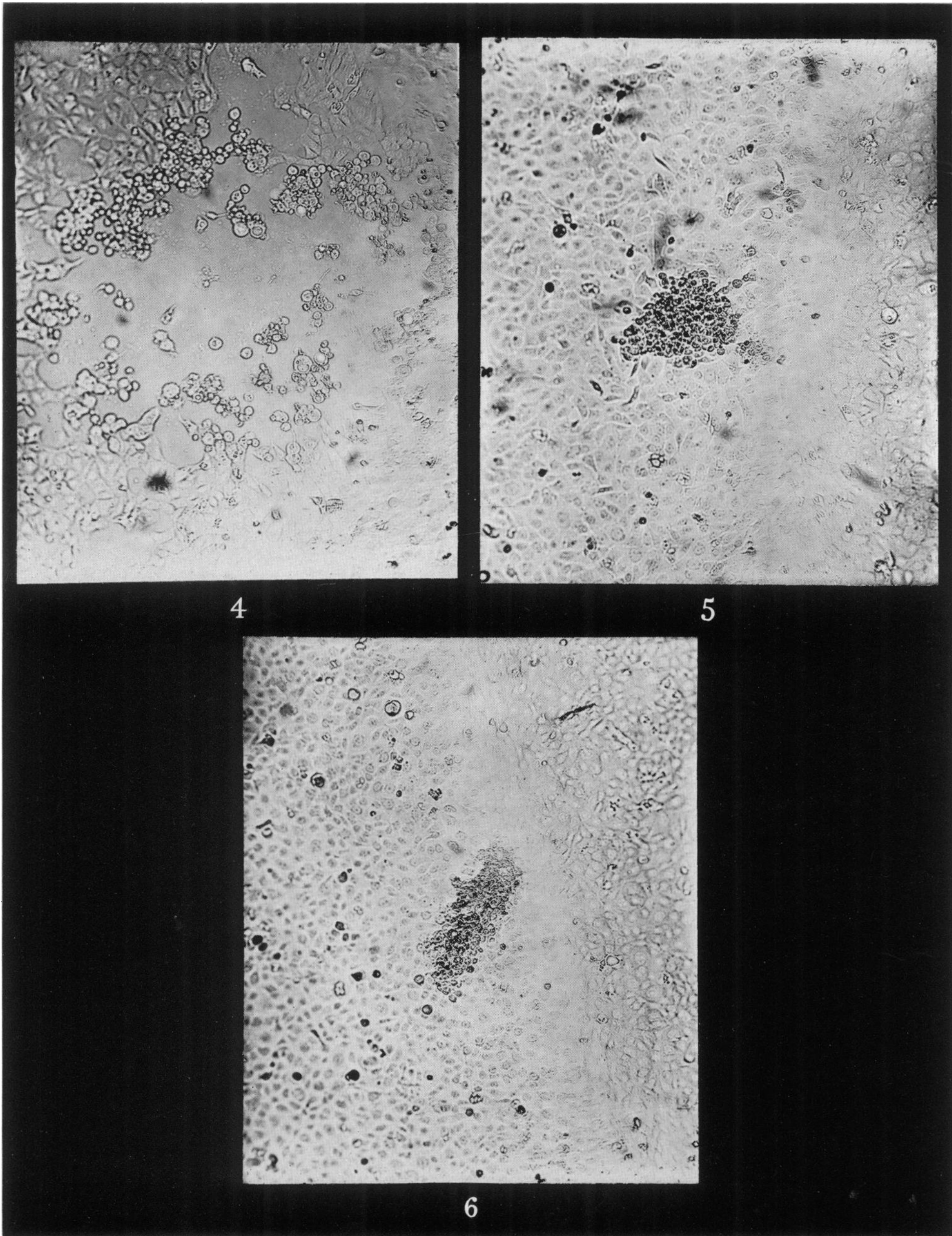
FIG. 4.—A neutralization test with zoster-infected cells as virus inoculum. The medium in this control amnion culture tube contained 5 per cent acute chickenpox serum. Note: cells photographed in the tube. × 36.

FIG. 5.—The same neutralization test as in Fig. 4 but the medium contained 5 per cent convalescent chickenpox serum (W.E.). Note the restriction in size of the lesion. × 36.

FIG. 6.—The same neutralization test as in Fig. 4 but the medium contained 5 per cent convalescent zoster serum (E.F.). Again there is marked restriction in size of the lesion. × 36.



Taylor-Robinson.


4
5
6

had been removed. Each tube then contained 20 per cent human serum. Two culture tubes were used for each serum tested. Four control tubes containing 20 per cent horse serum in place of the human serum were also set up. After incubation at 37° for 5 days the number of focal lesions in each tube was counted. The results are shown in Table II. Unfortunately a control human serum, from a child known not to have had chickenpox, caused non-specific degeneration of the amnion cells.

TABLE II.—*The Result of a Neutralization Test Using Zoster Vesicle Fluid* (*ZTA.*)

Patient	Duration of rash when serum taken (days)	Number of focal lesions in each of two culture tubes
		Zoster sera
C. B—	2	0, 1
	23	0, 0
S. F—	3	0, 4
	24	0, 0
C. H—	3	20, 10
	25	0, 0
		Chickenpox sera
W. I—	2	96, 100
	25	0, 1
W. E—	1	168, 176
	24	0, 0
W. H—	2	68, 70
	25	12, 10
I. T—	2	268, 180
	23	0, 0
Control tubes.	Horse serum only	700*, 650*, 600*, 800*

*Only very approximate count because of the number of lesions.

It may be seen that there is a very marked reduction in the number of focal lesions even with acute chickenpox serum. Because of the absence of the control with non-immune human serum, it is uncertain whether this result was due to the non-specific effect of human serum or to its antibody content or indeed the result of both. However, it would seem that acute zoster sera show greater neutralizing activity than chickenpox sera taken at the same interval after the onset of the rash. Both chickenpox and zoster convalescent sera at this dilution produced almost complete neutralization. Similar results were obtained in a further experiment with another zoster vesicle fluid.

In subsequent tests a simpler technique was used. Inactivated sera in 0·05 ml. quantities were placed in tube cultures of amnion cells from which the medium had been removed. Zoster vesicle fluid (ZTO.), diluted with Hanks' solution, was then added in 0·05 ml. quantities to each tube. The tubes were rocked 30 times to ensure mixing and left at room temperature for 30 min. Then 0·9 ml. of tissue culture medium (without horse serum) was added to each to give a final concentration of 5 per cent human serum. The tubes were again rocked several times, and incubated at 37°. Focal lesions were counted after 4 and 8 days ; the results are shown in Table III. Again there is good cross neutralization. Similar results were obtained in another test made in this way with a zoster vesicle fluid.

TABLE III.—*The Result of a Neutralization Test Using Zoster Vesicle Fluid* (*ZTO.*)

Patient		Duration of rash when serum taken (days)		Number of focal lesions in each of two culture tubes: Result on 4th day		Result on 8th day	
				Zoster sera			
S. A—	.	4	.	0,	0	4,	1
		25	.	0,	1	0,	2
				Chickenpox sera			
S. D—	.	2	.	28,	42	100,	110
		23	.	1,	0	2,	4
W. I—	.	2	.	27,	22	94,	80
		25	.	0,	0	1,	1
J. J. B—	.	3	.	38,	70	85,	100+
		120	.	0,	1	3,	2
Control tubes.		Horse serum only	.	40,	44	100+,	100+

Because only small amounts of vesicle fluid could be obtained, sera were not tested in serial dilutions. Nevertheless, the reduction in the number of focal lesions is such that the results are obviously significant. In all, 7 paired zoster sera and 9 paired chickenpox sera were used in neutralization tests with virus from 4 different zoster vesicle fluids. In contrast to acute chickenpox sera, acute zoster sera taken at comparable times from the onset of the eruption almost completely neutralized zoster virus. All convalescent chickenpox sera neutralized zoster virus, some of them as completely as did convalescent zoster sera.

Great difficulty was encountered in obtaining sufficient infective chickenpox vesicle fluid to test even a small number of sera. However, using the second technique described above, 2 tests were performed, Table IV shows the results obtained with CUN. and CGS. chickenpox fluids.

TABLE IV.—*The Result of Neutralization Tests Using Chickenpox Vesicle Fluids*

Patient		Duration of rash when serum taken (days)		Number of focal lesions in each of two culture tubes: Chickenpox fluid (CUN.)		Chickenpox fluid (CGS.)	
				Zoster sera			
C. H—	.	3	.	—,	—	0,	0
		25	.	—,	—	0,	0
M. M—	.	5	.	0,	0	0,	0
		26	.	0,	0	0,	0
				Chickenpox sera			
P. D—	.	4	.	2,	0	0,	1
		25	.	0,	0	0,	0
F. U—	.	1	.	9,	7	10,	9
		25	.	0,	0	0,	0
Control tubes.		Horse serum only	.	20,	—*	15,	14

— Sera not tested.
—* Non-specific degeneration of amnion cell sheet.

Because of the small number of lesions in the control tubes, these results are less significant than those with zoster vesicle fluid. Even so, it would appear that cross neutralization has occurred.

Tests with suspensions of infected amnion cells as source of virus

Because of the difficulty of obtaining sufficient infective vesicle fluid, from chickenpox cases particularly, a few tests were made using infected amnion cell suspensions.

One test was made by the second method used in the tests with the zoster vesicle fluids except that the concentration of test serum in each tube was 20 per cent. The cell suspension (chickenpox strain CDT.) was obtained by treatment of a single tube culture with 5 ml. of 0·25 per cent. Difco trypsin for 10 min. at 37°. The cells were dispersed as much as possible with a Pasteur pipette and then centrifuged at 2000 r.p.m. for 10 min. The supernatant trypsin was poured off and the cells resuspended in Hanks' solution served as virus inoculum. The sera were those used in the experiment recorded in Table IV. The results are shown in Table V.

TABLE V.—*The Result of a Neutralization Test Using Chickenpox-infected Amnion Cells as Virus Inoculum*

Patient	Duration of rash when serum taken (days)	Number of focal lesions in each of 2 culture tubes: Result on 3rd day	Result on 6th day
		Zoster sera	
C. H—	3	16, 6	33, 22
	25	4, 3	12, 12
M. M—	5	12, 10	44, 50
	26	1, 1	12, 18
		Chickenpox sera	
P. D—	4	12, 23	28, 32
	25	1, 0	5, 5
F. U—	1	—*, —*	—*, —*
	25	13, 3	26, 8

—* Non-specific degeneration of amnion cell sheet.

TABLE VI.—*The Result of a Neutralization Test with Zoster Vesicle Fluid (ZPQ.) and Sera from Individuals with a Past History of Chickenpox*

Patient	Time since chickenpox	Number of focal lesions in culture tubes: Result on 5th day	Result on 10th day
J. R—	40 yrs.	4, 4	8, 10
M. D—	18 ,,	0, 1	0, 1
D. E—	15 ,,	6, 5	8, 7
B. K—	13 ,,	2, 2	3, 2
T. I—	13 ,,	0, 0	0, 0
J. J. B—	17 weeks	0, 0	0, 0
D. T—	11 ,,	0, 2	1, 3
J. J. B—	3 days	31, 37	61, 70
F.M. convalescent zoster serum		0, 0	1, 0

Unfortunately, no horse serum control was set up and the tubes containing acute serum F.U., which might have served as negative controls, showed non-specific degeneration of the amnion cells. Nevertheless, there was a definite suggestion, both in the above test and in another with zoster-infected amnion cells, that chickenpox and zoster convalescent sera, although failing to effect complete neutralization, reduced the number of focal lesions. This result confirms the findings of Weller and Witton (1958).

In some experiments convalescent serum did not markedly reduce the number of focal lesions in comparison with a control acute chickenpox serum. But with the convalescent sera a striking reduction in the size of the lesions was observed. This is illustrated in figures 4, 5 and 6. The size of the lesions produced by zoster virus (strain ZPE., first passage) is inhibited equally by both zoster and chickenpox convalescent sera.

The persistence of neutralizing antibody after chickenpox

Sera from 7 persons with a past history of chickenpox were tested against zoster vesicle fluid. As controls, an acute chickenpox serum and a convalescent zoster serum were included. The acute chickenpox serum had been shown by previous tests to be almost devoid of antibody (JJB., Table III). The results of the test are shown in Table VI. It will be seen that neutralizing antibody persists in the serum for many years after a clinical attack of chickenpox. This contrasts with our finding that with the technique used complement-fixing antibody was not usually detectable in sera taken some months after the illness (Taylor-Robinson and Downie, 1959).

DISCUSSION

The results recorded above have served to confirm the pioneer studies of Weller on the propagation in tissue culture of virus from chickenpox and zoster cases (Weller, 1953 ; Weller, Witton and Bell, 1958). Although the technique differed somewhat from that of Weller in that stationary cultures of human amnion cells were used for most of the experiments reported here, the cytopathic changes observed were similar. No differences in behaviour were noted between strains derived from zoster cases and those from chickenpox. The same difficulty in obtaining cell-free infective virus from infected cultures was encountered, although on one occasion a small amount of such virus was obtained. Gold and Robbins (1958) also found that, in some experiments, infection of tissue cultures was achieved by means of cell free culture material.

The difficulty of demonstrating virus in the fluid phase of tissue culture is in sharp contrast to the finding with vesicle fluids. In such fluids virus appears to be, for the most part, extracellular ; cell-free filtrates of such fluids may, on occasion, have an infective titre almost as high as the unfiltered fluid. The presence of such free virus in vesicle fluid and presumably in the exudate from lesions on mucous membranes probably accounts for the high infectivity of varicella cases.

The absence of demonstrable infective virus in the fluid phase of the cultures presumably accounts for the focal nature of the lesions in tissue culture. However, the fact that immune serum in the medium may limit the number and size of lesions produced by infective cell suspensions suggests that some inhibition of spread of virus from cell to cell is effected by antibody. It would seem that virus

must pass extracellularly from cell to cell for such neutralization to take place. The difficulty of demonstrating virus in the fluid phase of tissue cultures suggests that the amount released into the medium is small or that it is unusually labile and rapidly undergoes inactivation. Electron micrographs of tissue cultures infected with varicella virus show virus particles apparently outside as well as within cells (Tournier *et al.* 1957).

Virus in vesicle fluid, because of its extracellular position, is more effectively neutralized in tissue culture by the immune serum in the medium. In tests using vesicle fluid as inoculum complete neutralization was effected both by convalescent chickenpox and convalescent zoster sera. As in the tests for complement-fixing and precipitating antibodies, there was complete cross reaction between sera from the two clinical conditions against virus from either entity. However, in the neutralization experiments, dilutions of sera were not tested. Consequently it has not been shown that convalescent zoster sera have a higher concentration of neutralizing antibody than convalescent chickenpox sera; such a difference was found in relation to complement-fixing and precipitating antibody. In the experiments of Weller and Witton (1958) and also in this work the antibody content of acute zoster sera was usually greater than that observed in chickenpox sera taken at the same time in the eruptive phase of illness.

The evidence from laboratory studies now strongly supports the epidemiological evidence that chickenpox and zoster are due to the same virus. The results of antibody tests reported in the present series of papers, and particularly the earlier and greater antibody response observed in zoster cases, support the view that zoster is a second clinical manifestation of infection with varicella virus in persons whose immunity from the primary infection, whether clinical or subclinical, has waned.

SUMMARY

Virus from 21 cases of zoster and 22 cases of chickenpox was grown in tissue cultures of human fibroblasts or human amnion cells. In sheets of the latter cells, the lesions produced are more circumscribed and more readily counted and for this reason amnion cell cultures were preferred for neutralization tests.

The lesions produced were focal in character and infection spread by involvement of contiguous cells as described by Weller. The changes produced by virus from chickenpox vesicle fluid were indistinguishable from those produced by virus from zoster. Demonstrable virus remained for the most part within the cells and only occasionally was a small amount of virus found in the culture fluid. In vesicle fluid, virus was mostly extracellular and survived for many months storage at $-70°$. Suspensions of infected tissue culture cells, on the other hand, were not found to be infective after storage at this temperature for more than a few days.

Virus in vesicle fluid was more effectively neutralized in tissue cultures by immune serum than was virus in suspensions of infected tissue culture cells. Paired sera from 7 cases of zoster and paired sera from 9 cases of chickenpox were used in neutralization tests with vesicle fluid virus. Both zoster and chickenpox convalescent sera in the culture medium produced almost complete neutralization of virus from zoster or chickenpox vesicle fluid.

Although the number of focal lesions was not always reduced in neutraliza-

tion tests carried out with infected amnion cell suspensions, there was reduction in the size of the lesions and this was sometimes very striking.

Sera from individuals who had suffered from chickenpox many years before showed significant neutralizing power although they had previously been shown to have no complement-fixing antibody.

REFERENCES

BLANK, H., CORIELL, L. L. AND SCOTT, T. F. McN.—(1948) *Proc. Soc. exp. Biol. N.Y.*, **69**, 341.
CHEATHAM, W. J.—(1954) Quoted by Enders, J. F., and Peebles, T. C. *Ibid.*, **86**, 277.
FELLER, A. E., ENDERS, J. F. AND WELLER, T. H.—(1940) *J. exp. Med.*, **72**, 367.
FOGH, J. AND LUND, R. O.—(1957) *Proc. Soc. exp. Biol. N.Y.*, **94**, 532.
GOLD, E. AND ROBBINS, F. C.—(1958) *Virology*, **6**, 293.
GOODPASTURE, E. W. AND ANDERSON, K.—(1944) *Amer. J. Path.*, **20**, 447.
TAYLOR-ROBINSON, D. AND DOWNIE, A. W.—(1959) *Brit. J. exp. Path.*, **40**, 398.
TOURNIER, P., CATHALA, F. AND BERNHARD, W.—(1957) *Pr. méd.*, **65**, 1230.
WELLER, T. H.—(1953) *Proc. Soc. exp. Biol. N.Y.*, **83**, 340.—(1958) 'The Harvey Lectures', 1956–1957. New York (Academic Press, Inc.), p. 228.
Idem AND STODDARD, M. B.—(1952) *J. Immunol.*, **68**, 311.
Idem AND WITTON, H. M.—(1958) *J. exp. Med.*, **108**, 869.
Iidem AND BELL, E. J.—(1958) *Ibid.*, **108**, 843.
YOUNGNER, J. S.—(1954) *Proc. Soc. exp. Biol. N.Y.*, **84**, 570.
ZITCER, E. M., FOGH, J. AND DUNNEBACKE, T. H.—(1955) *Science*, **122**, 30.