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Cell-free varicella–zoster virus in tissue culture

By ANNE E. CAUNT and D. TAYLOR-ROBINSON*
The Department of Bacteriology, University of Liverpool

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The cultivation of varicella–zoster (V-Z) virus in human tissue cultures was first reported by Weller & Stoddard (1952) and by Weller (1953). Infective virus was not detected in the fluid phase of cultures and serial propagation of virus could only be achieved by transfer of infected cells (Weller & Witton, 1953; Weller, Witton & Bell, 1958). This relationship between virus and tissue-culture cells was subsequently observed by several workers (see Downie, 1959). Weller (1958) also failed to obtain virus from infected cells by various disruptive procedures. However, Gold & Robbins (1958) reported briefly that they had produced a cytopathic effect in serial cultures of human amnion or monkey kidney cells by the use of cell-free tissue-culture material. Taylor-Robinson (1959) found that much of the infective virus in zoster vesicle fluids was cell-free in marked contrast to the absence of virus in varicella and zoster tissue-culture fluids. He mentioned, however, that free infective virus had, on occasions, been obtained by ultrasonic treatment of infected amnion cells. Furthermore, the presence of convalescent varicella or zoster serum in the fluid phase of infected tissue cultures inhibited the spread of focal lesions, indicating that infective virus was not entirely intracellular. Caunt (1963) has recently described experiments in which infective virus was liberated from infected human thyroid tissue cultures.

The first section of this paper describes this work in more detail. In thyroid cultures higher titres are obtained than in human amnion, virus can be detected in cell-free fluid and considerable amounts can be liberated from the cultured cells by sonic disintegration.

Our own and other workers' failure to find cell-free virus in earlier experiments suggested that virus released from cells in culture might have been inactivated at 37° C. before adsorption to uninfected cells. In Section II experiments to test this possibility are recorded and the possible role of interferon has been investigated.

MATERIALS AND METHODS

Viruses

Vesicle fluids from varicella and zoster patients were collected in tissue-culture maintenance medium and either used immediately or stored at −70° C. Inoculation of tissue cultures and serial propagation by passage of infected cells was carried out as described previously (Taylor-Robinson, 1959).

* Present address: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda 14, Maryland, U.S.A.

Sera

The immune sera used in complement fixation and neutralization tests were convalescent zoster sera taken 2–3 weeks after the onset of the rash. A standard serum for complement fixation tests has been used in this laboratory for several years and is known to give high titres with potent vesicle-fluid or tissue-culture antigens.

Tissue cultures

Primary trypsin-dispersed cultures of human amnion cells were prepared by the method of Zitcer, Fogh & Dunnebacke (1955). About 5×10^5 cells per ml. were distributed in 1·0 ml. amounts to 6 in. $\times$ $\frac{5}{8}$ in. tubes and in 20 and 35 ml. quantities to 6 and 12 oz. flat-sided medicine bottles respectively. Primary trypsin-dispersed cultures of human thyroid cells were prepared as described by Pulvertaft, Davies, Weiss & Wilkinson (1959) and were grown in similar containers.

Media

Amnion cultures were grown either in 10% inactivated horse serum and 10% tryptic digest broth in Hanks's saline with 0·03% bicarbonate or in 10% horse or lamb serum in Eagle's medium. Cultures were maintained in similar media but with the concentration of serum (horse, lamb or calf) reduced to 2 or 5%.

Thyroid cultures were grown in 5% calf serum in 199 medium with 0·06% bicarbonate and maintained in the same medium with 2% serum. All media contained 100 units/ml. of penicillin and 100 μg./ml. of streptomycin.

Complement fixation tests

Complement fixation tests were performed as described by Taylor-Robinson & Downie (1959).

Ultrasonic disintegration of cells

Bottle cultures of amnion cells with extensive cytopathic changes were trypsinized with 0·25% 'Difco' trypsin in phosphate-buffered saline, centrifuged at 1000 r.p.m. for 5 min. and the cell deposits resuspended in growth medium. The cells from a 6 oz. bottle were usually resuspended in about 1 ml. of medium. These cells, in a container surrounded by crushed ice, were disrupted with an M.S.E. Mullard ultrasonic disintegrator. The vibrator probe of stainless steel had end diameters of $\frac{3}{4}$ in. and $\frac{3}{8}$ in. and a velocity increase ratio of 4:1.

Infected thyroid cells were usually removed from the glass with 0·02% versene in phosphate-buffered saline but sometimes 0·25% 'Difco' trypsin was used as well. Subsequent treatment was the same as that for amnion cells.

Filtration

In some instances tests for the presence of cell-free infectious virus were made by filtration of material after ultrasonic disintegration of cells. Filtration was through 'Oxoid' membranes with an average pore diameter of 0·5–1·0 μ. The integrity of

the membranes was checked after each experiment by the filtration of *Chromobacterium prodigiosum* or *Escherichia coli* cultures; these filtrates were invariably bacteriologically sterile.

Ultracentrifugation

In some cases virus preparations were concentrated by centrifugation in a Spinco Model L centrifuge, Rotor No. 40 at 12,500 r.p.m. (14,000 *g*) for 30 min.

RESULTS

Section I

Comparison of human amnion and thyroid cells for the cultivation of V-Z virus

Human thyroid tissue cultures have been found to be more sensitive for the isolation of V-Z virus from vesicle fluids and to give higher titres in the titration of infected cell suspensions than human amnion cultures (see Table 1).

Table 1. *Comparison of the sensitivity of human amnion and human thyroid tissue cultures for the isolation and titration of V-Z virus*

Virus preparation	Number of focal lesions developing per culture tube of: Amnion	Thyroid
Vesicle fluid virus		
McG Varicella	0, 0	8, 3
Ho Zoster	31	63
Be Zoster	0, 0	40, 33
Mi Zoster	0, 0, 2, 0	6, 9, 9, 5
Infected cell suspension		
Mo Varicella 7th pass in thyroid	201, 210	400
Mo Varicella 9th pass in amnion	100, 68	323, 272

Human thyroid cultures infected with V-Z virus also appear to be a better source of soluble complement-fixing antigen than similar cultures in human amnion. The tissue-culture fluids from the latter require 3- to 10-fold concentration before they are useful antigens (Caunt, Rondle & Downie, 1961) but the unconcentrated fluids from infected thyroid cultures can be prepared and used in the following way. Cultures are inoculated heavily with V-Z virus (i.e. at least 500 infective units in a 6 oz. bottle) and, after 3–4 days when cytopathic changes are obvious, the normal maintenance medium is replaced by medium 199 with 0·09 % sodium bicarbonate and no serum. This medium is harvested after a further 5–7 days and may be replaced and a second harvest obtained if the cells are still viable. These fluids can then be used unconcentrated, or in some cases diluted up to 1/4, as antigen in complement fixation reactions (Table 2).

The absence of virus from the fluid phase of amnion tissue cultures

This phenomenon has been confirmed many times during the course of routine subculture. Occasionally the medium from bottle cultures of amnion cells showing

very extensive cytopathic changes has been centrifuged at 3000 r.p.m. for 10 min. and the top portion of the supernatant inoculated into amnion or thyroid cultures. Although a few focal lesions have sometimes occurred in the inoculated cultures, it is difficult to exclude the possibility that these are the result of virus within intact cells, or cell fragments, which did not sediment. If virus is free then it is present in amounts too small to be demonstrable in filtrates.

Table 2. *Complement fixation with antigens prepared in human amnion and human thyroid cells*

Antigen	Concentration or dilution	Dilution of zoster convalescent serum 1/16	1/32	1/64	1/128	Control (no serum)
203* (amnion)	× 10 concentration	+ + + +	+ + + +	+ + + +	+ + + +	−
	× 5 concentration	+ + + +	+ + + +	+ + +	+ +	−
225† (thyroid)	Unconcentrated	+ + + +	+ + + +	+ + + +	+ + + +	−
	½ dilution	+ + + +	+ + + +	+ + + +	+ + + +	−
	¼ dilution	+ + + +	+ + + +	+ + +	+ +	−

* Antigen 203 from Mi Zoster 7th passage in amnion cells maintained in 50% bovine amniotic fluid in Hanks's saline.

† Antigen 225 from Be Zoster 8th passage in thyroid cells maintained in medium 199.

+ + + +, complete fixation; + + +, + +, 25, 50% lysis; −, complete lysis.

The repeated subculture of V-Z virus in amnion cell cultures has not resulted in the appearance of virus in the fluid phase. Several strains of virus were subcultured for 10 or more passages and the varicella strain C.D.T. which was previously reported (Taylor-Robinson, 1959) to have undergone 33 subcultures has now been through 109 subcultures without infective virus being detected in the tissue-culture medium.

Presence of free virus in the fluid phase of thyroid tissue cultures

In thyroid tissue cultures 10–14 days after inoculation with V-Z virus, very small, presumably secondary, focal lesions occur in addition to the primary foci, which are very much greater in diameter by this time. This suggested the presence of infective virus in the culture fluids and with several virus strains this has now been demonstrated. The strains were derived from both varicella and zoster cases and with a heavy inoculum free virus was first demonstrated after 3 days, but continued to be released until the cells disintegrated. The titre of virus in the fluid was not high but the virus concentration could be increased by ultracentrifugation. This virus was filterable and was neutralized by immune serum (Table 3) in the same way as virus from vesicle fluid.

Effect of ultrasonic disintegration of infected tissue cultures

Numerous experiments were carried out in an endeavour to release virus from infected amnion and thyroid cells. Preliminary experiments showed that ultrasonic treatment of vesicle fluid halved the virus content for each minute up to 2 min. (Table 4). We assumed that, if similar virus was liberated from tissue-

Table 3. *V-Z virus in thyroid tissue-culture fluids*

	Number of focal lesions developing from 1 ml. of	Virus strain McC23	Virus strain McC24
A	Supernatant fluid after centrifuging 1000 r.p.m. 5 min.	46, 42	6, 4, 5
B	Fluid from *A* after filtration	2, 6	2, 1, 1
C	Fluid from *A* concentrated × 70 by ultracentrifugation	NT	100, 110
D	Fluid from *C* + 0·1 ml. 1/5 immune serum	NT	0, 0
E	Fluid from *B* + 0·1 ml. 1/5 immune serum	0, 0	NT

NT, Not tested.

Table 4. *Effect of ultrasonic treatment on virus in zoster vesicle fluids*

Zoster vesicle fluids	Number of focal lesions produced in amnion cultures by vesicle fluids after ultrasonic treatment for 0 sec.	15 sec.	1 min.	2 min.	5 min.	10 min.
ZHA	141	116	NT	NT	NT	NT
ZHO	31	NT	17	8	3	2

culture cells, half of it also would survive 1 min. of treatment and some would survive 2 min. When infected amnion cells were subjected to this treatment for various periods up to 2 min., titration of the cell debris showed that there was a marked reduction in infectivity compared with the original infected cell suspension (Table 5). However, on several occasions filtrates of the disrupted cell suspension contained small quantities of infective virus. The amount of virus obtained by this procedure was too small to be of practical value as a source of cell-free virus in the laboratory.

Table 5. *Effect of ultrasonic disintegration of infected human amnion cells*

Virus strain and passage	Number of focal lesions produced in amnion cultures per ml. of: Original infected cell suspension	Suspension of cell debris after treatment for 15 sec.	30 sec.	45 sec.	1 min.	1½ min.	2 min.
Th (Varicella) 62	500	13	1	0	NT	NT	NT
	54	0	NT	(2)†	NT	NT	NT
	500	NT	17 (1)†	NT	NT	NT	NT
Mo (Varicella) 9	3000	50	25	15	20	40	NT
Wr (Varicella) 4*	580	NT	NT	NT	NT	NT	55 (5)†

* In this experiment the titration was in thyroid cultures.
† Figures in brackets = number of focal lesions per ml. of material after filtration.
NT, Not tested.

When thyroid cultures were similarly treated there was little loss of infectivity. If the cell debris suspension was centrifuged at 3000 r.p.m. for 5–10 min. some of the infectivity was lost with the sediment but filtration of the supernatant had little effect on the virus titre (Table 6, first line). Supernatant fluids with titres of

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10^4–10^5 infectious particles per ml. have been obtained and this has proved to be a practical method of obtaining virus for use in neutralization tests. Such preparations are also excellent complement-fixing antigens and one such as that from J 9 in Table 6 when used diluted 1/10 gave complete fixation with 1/256 dilution of the convalescent zoster serum used in Table 2. This cell-free virus has also been stored at −63° C., when some loss of infectivity occurs. It is not yet known whether this loss is related to the length of storage but in the case of vesicle-fluid virus a similar loss occurs; the extent of this loss varies but has no apparent relationship to length of storage.

Table 6. *Effect of ultrasonic disintegration of infected human thyroid cells*

	Titre expressed as number of focal lesions produced in thyroid culture per ml.					
					Materials after storage at −63° C.	
Virus strain and passage	Original infected cell suspension	After 2 min. ultrasonic treatment	Supernatant after centrifuging treated material	Filtrate of treated material	Titre	Days of storage
McC (Zoster) 8	2×10^5	9×10^4	2×10^4	2×10^4	$1{\cdot}3 \times 10^3$*	8
Mo (Varicella) 5	4×10^3	NT	NT	2×10^3	1×10^2*	14
Mo (Varicella) 7	$2{\cdot}4 \times 10^4$	3×10^4	NT	5×10^3	NT	—
J (Varicella) 9	2×10^5	NT	$1{\cdot}6 \times 10^5$	NT	6×10^4†	10
					$2{\cdot}6 \times 10^4$†	84

* Filtrates.
† Unfiltered supernatant fluids.

Section II

In considering the low titre or complete lack of free virus in the fluid phase of tissue cultures, it seemed possible that under normal tissue-culture conditions small amounts of virus might be released but be inactivated at 37° C. before they can be readsorbed to normal cells. The following experiments were carried out to test this possibility.

Rate of inactivation of virus at 37° C.

Virus was diluted in tissue-culture maintenance medium and seven 2 ml. samples of this material were incubated at 37° C. At various times up to 15 hr. successive samples were inoculated in 1 ml. amounts into thyroid cultures. The results in Table 7 show that there was no appreciable inactivation of vesicle-fluid virus within 9 hr. and little within 15 hr., although with virus grown in tissue culture only about 60 % survived for 9 hr.

Rate of adsorption of virus to tissue-culture cells

The rate of adsorption of virus to amnion and thyroid cells in tissue culture was tested using either varicella or zoster vesicle fluid or virus obtained by the disintegration of infected thyroid cells. In one of these experiments 0·25 ml. of zoster vesicle fluid was diluted to 7 ml. with tissue-culture maintenance medium and inoculated in 0·1 ml. quantities into 7 tube cultures of amnion cells; these cultures

Table 7. *Rate of inactivation of virus at* 37° C.

Virus	Time (hr.)	Number of focal lesions/ml. of virus suspension	Average number/ml.	Survival (% of count at 0 hr.)
Varicella vesicle fluid	0	75, 81	78	100
	3	97, 78	87	—
	6	62, 87	74	94·9
	9	73, 71	72	92·3
	12	63, 52	57	73·1
	15	46, 51	48	61·5
Varicella 9th pass from thyroid cells	0	108, 111	109	100
	3	109, 81	95	87·1
	6	55, 54	55	50·5
	9	67, 59	63	57·8

are referred to as 'original tubes'. Immediately after inoculation the fluid was removed from one of the original tubes and placed in a fresh tube (*A*). Then the original tube (*O*) was rinsed with 1 ml. of fresh medium which was rocked 30 times before it was removed to a fresh culture (*B*); 1·0 ml. of fresh medium was then added to the original tube. These three tubes, and the remaining 6 original tubes, were then incubated at 37° C. This procedure of removing the medium and rinsing out the tube was carried out on successive cultures at 3-hourly intervals up to 15 hr. and the seventh culture was left as a control. The results recorded after 5 days' incubation at 37° C. are shown in Table 8 (*a*). The amount of virus adsorbed (tube *O*) and the amount removed from the cultures at each period of time was known (tubes *A* and *B*), so that the percentage of virus fixed at each time interval could be calculated. The results of a similar experiment using varicella vesicle fluid and thyroid cells are shown in Table 8 (*b*), and of using virus from thyroid cultures and thyroid cells in Table 8 (*c*). It can be seen that in these experiments 93, 85 and 84 % respectively of the virus inocula were adsorbed in 9 hr. and the virus which was not adsorbed remained infective for fresh cultures. In experiment (*b*) of Table 8 a duplicate set of original tubes was set up and a zoster convalescent serum was added at the various time intervals to neutralize free virus. In these tubes the maximum number of infected foci was attained by 9 hr., thus confirming that the virus inoculum was adsorbed within that time. It is noticeable, however, that the number of infected foci developing under immune serum was less than those in tubes without immune serum. This suggests that, although the virus was adsorbed to the cells within 9 hr. and could not then be washed off, some of it remained at the surface of the cells and could be neutralized by immune serum even after 12 or 15 hr.

These results, together with the observations on inactivation of the virus at 37° C., show that most of the virus inoculum is adsorbed to the cells within 9 hr. and that little inactivation occurs within this period. This suggests that if virus is liberated from infected cells in tissue culture it is unlikely to be inactivated at the temperature of incubation before being adsorbed to normal healthy cells.

Table 8. *The rate of adsorption of virus to tissue-culture cells*

Virus	Tissue culture	Time (hr.)	Number of focal lesions/tube *O*	*A*	*B*	Total number of focal lesions (*T*)	% virus adsorbed ($O/T \times 100$)	Duplicate tube + immune serum
(*a*) Zoster vesicle fluid	Amnion	0	6	311	11	328	2	—
		3	190	140	21	351	54	—
		6	155	86	20	261	60	—
		9	430	14	17	461	93	—
		12	412	5	2	419	98	—
		15	410	1	2	413	99	—
		Virus control	415	—	—	415	—	—
(*b*) Varicella vesicle fluid	Thyroid	0	2	35	5	42	5	0
		3	38	40	4	82	46	22
		6	55	10	1	66	83	33
		9	58	10	0	68	85	46
		12	59	2	2	63	94	36
		15	73	1	1	75	97	42
		Virus control	78	—	—	78	—	—
(*c*) Varicella 9th pass from thyroid cells	Thyroid	0	4	128	8	140	2·9	—
		3	87	86	22	195	44·6	—
		6	137	30	10	177	69·5	—
		9	111	27	4	142	84·1	—
		Virus control	111	—	—	111	—	—

O, Original tube, incubated for time shown; *A*, medium removed from original tube after time shown, to a fresh culture; *B*, rinsings of original tube, removed to a fresh culture.

Attempts to demonstrate interferon production

A possible explanation of the inability to detect free infective virus in the fluid phase of infected amnion cultures, and for the absence of secondary foci, was that the infected cells produced interferon. We attempted to demonstrate the presence of interferon in amnion and thyroid cultures heavily infected with V-Z virus, i.e. cultures in which 75% or more of the cell sheet showed cytopathic changes. The medium from such cultures was centrifuged at 1000 r.p.m. for 5 min. and the supernatant was added to cultures of amnion or thyroid which were then incubated for 24 hr. at 37° C. Virus from vesicle fluid, or in one case from thyroid tissue cultures, was then added to some of the 'interferon-treated' cultures and to control cultures not so treated. The results of these experiments are shown in Table 9. There is no evidence of interferon production in any of the tests. The fluid from infected cultures was also tested for interferon in chick embryo fibroblast tissue cultures using Sindbis as indicator virus and no interferon was demonstrated. It therefore appears that if interferon is produced in infected cultures there is not enough to account for the absence of free virus or of secondary focus formation.

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Table 9. *Attempts to demonstrate interferon production in infected tissue cultures*

Methods used to produce interferon				Test for presence of interferon				
						Number of focal lesions per culture		
Tissue cultures	Inoculum for cultures	Period of incubation (days)	Time of contact of medium used as 'interferon' (days)	Virus	Tissue culture	Untreated culture + virus	'Interferon'-treated culture + virus	'Interferon'-treated culture (no virus)
Amnion	Zoster 11th pass	13	7–13	Zoster V-F	Thyroid	32	29	0, 0
Thyroid	Varicella 1st pass	20	13–20	Zoster V-F	Thyroid	26	55	0, 0
Amnion	Varicella 11th pass	7	4–7	Varicella V-F	Amnion	72	147, 190	9, 4
Amnion	Varicella 11th pass	7	4–7	Varicella V-F	Amnion	72	69	1, 3
Amnion	Varicella 11th pass	7	4–7	Varicella* 8th pass	Amnion	142	105, 85, 85	1, 3

* Virus prepared by ultrasonic disruption of infected thyroid cells.
V-F, Vesicle fluid.

DISCUSSION

The most striking aspect of our experiments has been the ability to obtain cell-free, infective virus in small quantities in the fluid phase of thyroid tissue cultures and in much larger amounts by the disintegration of such cultures. This is in marked contrast to our experience with amnion cells and that of previous investigators using amnion, human embryo fibroblasts and HeLa cells (Weller & Witton, 1953; Weller *et al.* 1958; Taylor-Robinson, 1959; Rapp & Benyesh-Melnick, 1963). Little or no free infective virus occurs in the fluid phase of infected amnion cultures and although small amounts can be released by ultrasonic disintegration of these cells much greater quantities (at least 100 times as much) are obtained by a similar treatment of thyroid cells.

Weller *et al.* (1958) suggested that the virus within tissue-culture cells exists in an '*immature*' *non-infective form and that death of the cell results in loss of this* virus. In both infected amnion and thyroid cells there is some loss of infectivity on disintegration of the cells but this loss is very much less in the case of thyroid cells. Even allowing for the fact that there may be greater viral synthesis in thyroid cells, a view which is supported by the greater production of soluble complement-fixing antigen by these cells than by amnion cells, it seems likely that the difference in the yield of free virus by the two cell systems is not a purely quantitative one and that a greater proportion of the virus in infected thyroid cultures is present in a complete or mature form. Some of this mature virus is in fact released into the supernatant fluid.

Tournier, Cathala & Bernhard (1957) observed particles with the electron microscope, presumably virus particles, outside as well as within human embryonic fibroblasts infected with varicella. The infectivity of these particles was not determined but the observation does suggest that virus is released from these infected cells. We have investigated the possibility that small amounts of virus might be liberated from amnion cells and that this virus would be inactivated at 37° C. before it could be adsorbed to healthy cells. Our observations indicate that this is unlikely to be the case. We have also failed to demonstrate an interferon-like substance in sufficient amounts to protect healthy cells in tissue culture. This is not surprising since foci continue to increase in size over many days in monolayers of amnion and if interferon were formed it might be expected to protect cells adjacent to the infected ones.

It has previously been noted (Weller *et al.* 1958; Taylor-Robinson, 1959) that the presence of zoster or varicella immune serum in the medium of tissue cultures does not prevent the development of infected foci in monolayers inoculated with infected cells, although it does neutralize vesicle fluid virus and, as we have shown, cell-free virus from tissue cultures. The foci which develop from infected cells under antiserum, however, are smaller in size, suggesting that spread of infection by extracellular virus, which can be affected by antiserum, normally does occur.

It is interesting to compare these findings with those relating to the growth of herpes simplex in HeLa cells (Stoker, 1959). In this work single infected HeLa cells were found to be capable of initiating foci of infection in HeLa cell monolayers

even in the presence of antiserum, although they did not release infective virus if they were grown in isolation in microdrops. It was postulated that virus was present in the infected cells in a 'vegetative' state and that if released into the medium it lacked the power to penetrate new cells and therefore could not be detected. However, in monolayer cultures this vegetative virus was able to spread, particularly by giant cell formation in which there was fusion of contiguous cells. Formation of giant cells is also a feature of the growth of V-Z virus in monolayers of amnion and thyroid but at the periphery of infected foci there are always some infected cells which retain their individual integrity. The virus must therefore be able to infect intact cells and spread is not simply accomplished by the fusion of cells.

Recently Slotnick & Rosanoff (1963), using the fluorescent antibody technique of Weller & Coons (1954), have shown that varicella antigen is present in the cytoplasmic strands which join the cells and it is possible that infection of adjoining cells occurs via these strands before the infected cell or giant cell separates completely from its neighbours.

Other recent work which may be relevant concerns the isolation of infectious DNA. Such DNA from polyoma virus is capable of initiating plaque formation in monolayer tissue cultures (Weil, 1961), and DNA from papilloma virus can produce papillomas in the skin of rabbits and is unaffected by concentrations of antiserum which completely inactivate papilloma virus suspensions (Ito, 1960).

It might be suggested that V-Z virus exists inside amnion cells as infectious DNA rather than as complete virus and that this can infect adjacent cells, but if it is released from the cell it combines with non-viral protein in the tissue culture medium and hence becomes non-infective. This would explain the loss of infectivity on disruption of the cells but does not explain the partial inhibition of spread of virus under antiserum since the DNA would not be neutralizable by immune serum. Little is known of the changes which take place in cells infected with V-Z virus and it is possible that further cytological and electron-microscopic studies might be helpful in elucidating the means of cell-to-cell transfer of infection and also in showing what differences there are between virus formation in thyroid and in other susceptible cells.

SUMMARY

Infected human thyroid tissue cultures have been found to yield free V-Z virus on ultrasonic disintegration and some virus is also present in the fluid phase of intact tissue cultures, while the yield of virus from infected human amnion cells is very low and no free virus is found in the fluid phase. Thyroid cultures are more susceptible to infection with V-Z virus, and the infected cultures are a better source of complement-fixing antigen, than are amnion cultures.

The lack of free virus in infected amnion tissue cultures cannot be attributed to protection by interferon. It also seems likely that if any infectious virus were released it would be more likely to be adsorbed to normal cells than to be inactivated, but as no secondary foci appear in amnion monolayers we conclude that infective virus is not released into the medium.

It is suggested that the virus in thyroid cells is present in a more complete form than that in amnion cells.

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