CLASSIC

PAPER

Classic paper: How monolayer cell culture transformed diagnostic virology: a review of a classic paper and the developments that stemmed from it[†]

John F. Enders, Thomas H. Weller and Frederick C. Robbins

Reviewed by Dr P. Mortimer*

Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London, NW9 5HT, UK

INTRODUCTION

The isolation of viruses from clinical specimens inoculated onto pre-formed monolayers of cells, grown on either glass or transparent plastic surfaces, has been a central plank of viral diagnostics for 50 years. So much is it now part of the routine of clinical virology that most practitioners of the art are hazy about or have forgotten its origins.

Like many really useful medical techniques *in vitro* virus propagation is a hybrid derived from various strands, and it owes its inception to several outstanding American scientists who flourished in the first half of the 20th century. They were, notably, Ross Harrison, Peyton Rous and John Enders. It evolved out of practical advances in cell biology such as the use of trypsin to disperse cells from solid tissue and the creation of continuous and semi-continuous cell lines; also out of the availability of antibiotics to maintain culture sterility. Virus isolation technique was then refined by the development of methods for recognising occult viral replication on monolayers, aided by skills acquired in passaging and maintaining the cultures long term. Consequently, within 10 years of the publication of the classic paper reproduced below, most of the readily cultivable animal viruses had been isolated and adapted to growth in cell culture. Since then the polymerase chain reaction has emerged as a rival

viral diagnostic technique, but it lacks the open-ended potential of monolayer culture as a means of virus detection. Future diagnostic practice will more likely see these two techniques being used in conjunction to achieve even faster and more sensitive virus isolation and identification.

I have chosen to review Enders, Weller and Robbins' description of poliovirus propagation in tissue culture as it is a pivotal paper in the mid 20th century access into the previously hidden world of human and animal pathogens that do not replicate on inert media. At a stroke, it also relieved the log jam in the diagnosis, epidemiological understanding and vaccinology of poliomyelitis. For this the authors were awarded a Nobel prize. Other contributions, before and after, were essential for bringing the *in vitro* culture of viruses within reach of even the humblest laboratory facility, and it is upon these that, following the text of Enders et al, I concentrate my discussion. This is not meant to detract from the classic paper, only to set it in its wider context.

CULTIVATION OF THE LANSING STRAIN OF POLIOMYELITIS VIRUS IN CULTURES OF VARIOUS HUMAN EMBRYONIC TISSUES¹

JOHN F. ENDERS, THOMAS H. WELLER,² and FREDERICK C. ROBBINS³

Research Division of Infectious Diseases, Children's Hospital, and Departments of Bacteriology, Comparative Pathology, and Pediatrics, Harvard Medical School, Boston

*Corresponding author: Dr P. Mortimer, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London, NW9 5HT, UK.
E-mail: philip.mortimer@hpa.org.uk

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¹Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

²Post doctorate Fellow of the U. S. Public Health Service.

³Senior Fellow in Virus Diseases of the National Research Council.

TABLE 1. Multiplication of Lansing poliomyelitis virus in tissues obtained from the extremities of human embryos

Culture set	No. of nutrient fluid changes prior to subculture		Day of incubation subculture done		Mouse LD ₅₀ of pooled fluids used to inoculate subcultures		Calculated dilution of original inoculum at time of subculture	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Original*	3	4	20th	20th	10 ^{-2.0}	10 ^{-1.57}	10 ⁻⁵	10 ^{-6.2}
1st subculture	2	2	19th	12th	10 ^{-1.57}	10 ^{-1.71}	10 ^{-8.8}	10 ^{-10.0}
2nd subculture	2	4	12th	20th	10 ^{-0.63}	10 ^{-1.34}	10 ^{-12.6}	10 ^{-16.2}
3rd subculture	3	...	16th	...	10 ^{-0.16}	...	10 ^{-17.7}	...

*The LD₅₀ of the suspension of mouse brain used as the inoculum in the first experiment was 10⁻²; that of the suspension employed in the second experiment was 10^{-1.01}.

An extraneural site for the multiplication of the virus of poliomyelitis has been considered by a number of investigators (2, 5). The evidence that this may occur is almost entirely indirect, although recent data indicate that Theiler's mouse encephalomyelitis virus as well as various mouse pathogenic poliomyelitis-like viruses of uncertain origin may multiply in nonnervous tissue (1, 3). Direct attempts by Sabin and Olitsky (4) to demonstrate *in vitro* multiplication of a monkey-adapted strain of poliomyelitis virus (MV strain) in cultures composed of certain nonnervous tissues failed. They obtained, however, an increase in the agent in fragments of human embryonic brain.

The general recognition that the virus may be present in the intestinal tract of patients with poliomyelitis and of persons in contact with them emphasizes the desirability of further investigation of the possibility of extra-neural multiplication. Accordingly, experiments with tissue cultures were undertaken to determine whether the Lansing strain of poliomyelitis virus could be propagated in three types of human embryonic tissues. The results are summarized here in a preliminary manner.

The technique was essentially the same as that recently described for the cultivation of mumps virus (6). The cultures consisted of tissue fragments suspended in 3 cc of a mixture of balanced salt solution (3 parts) and ox serum ultrafiltrate (1 part). Tissues from embryos of 2½ to 4½ months as well as from a premature infant of 7 months' gestation were used. These were: the tissues of the arms and legs (without the large bones), the intestine, and the brain. Each set of cultures included 4 or more inoculated with virus, and usually a similar number of uninoculated controls. The primary inoculum consisted of 0.1 cc of a suspension of mouse brain infected with the Lansing strain of poliomyelitis virus.⁴ The identity of the virus was verified by (a) the character of the disease it produced in white mice following intracerebral inoculation; and (b) its neutralization by specific antiserum.⁵ Subcultures were inocu-

lated with 0.1 cc of pooled *centrifuged* supernatant fluids removed from the previous set of cultures.

The procedure of cultivation differed from that usually followed by other workers in that the nutrient fluid was removed as completely as possible and replaced at periods ranging from 4 to 7 days. Subcultures to fresh tissue were prepared at relatively infrequent intervals, ranging from 8 to 20 days.

Two experiments have been carried out employing cultures composed chiefly of skin, muscle and connective tissue from the arms and legs. The findings in each have been essentially the same. In the first, a series of cultures has now been maintained for 67 days. During this interval, in addition to the original set, three successive subcultures have been made to fresh tissue and the fluids have been removed and replaced 10 times (Table 1). Assuming that at each change of fluid a dilution of approximately 1:15 was effected and that at the initiation of each set of cultures the inoculum was diluted 30 times, it has been calculated that the 10% suspension of infected mouse brain used as the primary inoculum had been diluted approximately 10¹⁷ times in the fluids removed from the third subculture on the 16th day of cultivation. These fluids, however, on inoculation into mice and monkeys, produced typical paralysis. Accordingly, since the mouse LD₅₀ of the original inoculum was 10⁻², it would appear that the increase in virus during the course of the experiment was at least of the order of 10¹⁵ times. During the 67-day period of cultivation a progressive decrease in mouse infectivity was recorded (Table 1). On the other hand, in the second experiment, mentioned above, the calculated increase in virus during a 52-day period is now of the order of 10¹⁶ times and no decrease in mouse infectivity has so far been observed.

The agent propagated in the first experiment continued to exhibit the principal characteristics of the Lansing strain during the period of cultivation, as indicated by the following observations: (a) fluids from each set of cultures produced paralysis and death in mice after intracerebral inoculation; (b) the agent present in the fluids of the second set of subcultures was neutralized by antiserum specific for the Lansing strain; (c) following intracerebral inoculation, the fluids from the third set of subcultures produced flaccid paralysis within 7 and 10 days,

⁴This strain was isolated by Dr. Charles Armstrong and obtained through the courtesy of Dr. S. D. Kramer.

⁵This serum was obtained through the courtesy of Dr. Isabel Morgan. It was prepared by hyperimmunization of rhesus monkeys with her strain of the Lansing virus.

respectively, in two rhesus monkeys. Microscopic examination of the spinal cords of these animals revealed lesions characteristic of poliomyelitis.

Cultures of intestinal tissue were prepared with fragments from the entire intestine of human embryos, except in one experiment in which jejunum of a premature infant was used. In the latter, the bacteria were eliminated in the majority of cultures by thorough washing of the tissue and by the inclusion in the original nutrient fluid of 100 units/cc of penicillin and of streptomycin.

In one experiment with embryonic intestine, which included two subcultures and 7 changes of nutrient fluid, the calculated dilution of the original inoculum was of the order of $10^{13.7}$ times. On the basis of the mouse LD_{50} of the original inoculum and that of the last supernatant fluid, it was calculated that the virus had increased about $10^{12.7}$ times. The identity of the agent thus cultivated in intestinal tissue has not yet been confirmed by neutralization tests or monkey inoculation, but it elicits a response in the mouse typical of the Lansing virus.

The cultures prepared with intestine of the premature infant have, so far, been maintained 17 days with 3 changes of nutrient fluid. Virus has been demonstrated, by mouse inoculation, in the fluids removed during the course of the experiment, including that of the 17th day. The calculated multiplication of the virus was approximately 10^3 times. This finding suggests that multiplication occurred in this tissue which, from the embryologic point of view, is more mature.

To compare the increase of virus in nervous tissue with that in tissue of the intestine and the extremities, cultures of embryonic brain were prepared. The multiplication of the virus in this medium has been comparable to that in the other types. Thus in one experiment carried out contemporaneously with the series of embryonic intestinal cultures mentioned above, the calculated multiplication of virus was of the order of 10^{12} times.

No evidence was obtained which indicated that an agent other than the Lansing strain of virus was propagated in any of the three types of tissue. Mouse infectivity tests for the presence of virus in the supernatant fluids of uninoculated control cultures were negative. Aerobic and anaerobic cultures of supernatant fluids yielded no growth of bacteria.

On microscopic examination of fragments of the three types of tissue, removed after about 30 days of cultivation, differences have been observed in cell morphology between those derived from inoculated and uninoculated cultures. Many of the fragments from uninoculated cultures contained cells which appeared to be viable at the time of fixation, as indicated by the normal staining properties of the nuclei and cytoplasm. In contrast, the nuclei of the majority of the cells in fragments from inoculated cultures showed marked loss of staining properties. Since the amount of material which has been studied is as yet relatively small, one cannot conclude that the changes observed in the inoculated cultures were caused by the virus.⁶

⁶We are indebted to Dr. Duncan Reid and members of the staff of the Boston Lying-in Hospital for providing the human embryonic tissues and to Dr. Alvin M. Pappenheimer for the preparation and examination of sections of tissue culture material.

It would seem, from the experiments described above, that the multiplication of the Lansing strain of poliomyelitis virus in the tissues derived from arm or leg, since these do not contain intact neurons, has occurred either in peripheral nerve processes or in cells not of nervous origin.

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COMMENTARY

Several aspects of this short paper should attract the modern reader. Firstly, no words are wasted, and the scene is set with only half a dozen references in support. Secondly, the 'message' of the paper (that poliovirus will grow outside the nervous system) is communicated within the first sentence and reiterated in the last. Thirdly, the question of whether *in vitro* virus multiplication as opposed to mere passive passage has been achieved is tackled face on, and convincingly dealt with. Finally, one sees at a technical level that the work must have been scrupulously done to obtain so satisfactory a result. Only as a throwaway line is the use of 'pen/strep' mixture mentioned, but subsequently this became a regular precaution in cell culture work.

It might also be worth noting, before attempting to set this classic paper in its historical context, that although reference is made to virological effects on the cultured cells these were not actually monolayer cell cultures; nor were kidney cells used, even though they were shortly found to be the most convenient tissue for the propagation of polioviruses. The experiments were done on human embryonic tissue from fetuses of 2½, 4½ and 7 months gestation. Whether, *de novo*, such uses of foetal tissue would receive ethical approval today is debatable; but if ends ever justified means, then here was an example of it.

Virchow, the doyen of nineteenth century cell pathology, is reputed to have said that '*die Methode ist Alles*' [1] and this is certainly true of the study of viruses. Today, no one can claim to know the virology who has not familiarised themselves with monolayer cell culture as a means of propagating viruses. It is a simple technique, but it would not have come about as quickly as it did without Enders, Weller and Robbins' classic paper of 1949. This showed that, *in vitro*, viruses might grow in cells for which they were apparently not tropic *in vivo*. By the end of the following decade monolayers of common cell lines were being prepared for viral diagnosis in laboratories on every inhabited continent.

The fact that viruses are obligate parasites of living cells was scarcely understood by the first generation of medical scientists to investigate the infectious diseases that had not yielded up their causative agents as bacterial isolates on inert growth media. By 1950 a few of these refractory filter-passing agents had been grown in the organs of susceptible laboratory animals (e.g. in a mouse's brain or an embryonated egg), but investigators had made little progress in propagating them *in vitro*. The growth of vaccinia virus in the tissue culture system described by the Maitlands [2] exemplified what *had* been achieved by then, but the very title of their paper suggests that they had not grasped the fact that tissue cultures needed to be viable for virus replication to occur. Perhaps only those few contemporaries who grew bacteriophages on lawns of bacteria appreciated that living host cells were required for virus propagation. If they did so understand, however, they didn't shout about it.

Fortunately others, not themselves microbiologists, were developing methods for culturing tissue *in vitro* [3]. This prepared the way for the rapid technical leaps forward around 1950 that enabled the ready propagation of viruses on living monolayers of cells. This monolayer cell culture gave a boost to virology quite as powerful as the one that bacteriology had received 70 years earlier when Koch and his associates introduced semi-solid growth media for the isolation of bacterial species.

At first, the monolayers were used to isolate familiar viruses such as polio, herpes simplex and vaccinia [4–6]; but within a decade a swathe of other known and previously unknown virus species had been isolated on cell monolayers [7]. Since the 1960s relatively few more human and animal viruses have been added to that list, but

cell culture remains an important tool for isolating emerging viruses and some other infectious agents, possibly in the future to include prions. Cell monolayers have also been an important substrate for detecting the presence of bacterial toxins such as those of *Clostridium difficile*, both in faecal specimens and from isolates of toxigenic bacteria from cases of pseudomembranous colitis and antibiotic-associated diarrhoea [8].

At first the work on diagnostic cell cultures was fuelled by the vast public funding and human effort invested during the 1930s and 1940s in overcoming poliomyelitis, with the US National Foundation for Infantile Paralysis being pre-eminent [9]. Later, research aims broadened out to embrace the diagnosis of other acute and chronic viral infections of man and animals. The references chosen here to represent this large field are necessarily selective, but they attempt to identify the main early contributions of cell culture to the development of vertebrate diagnostic virology.

A LONG HIATUS

One might first ask why the interval was so long from Ross Harrison's original description of a tissue culture (1907) and from Rous and Jones' first description (1916) of the use of trypsin to disperse cells and coax them into forming monolayers [10] to the first inoculations of monolayers with viral specimens in the early 1950s. The reasons were several, and foremost was the belief that viruses would only grow in the cells, and even only the relevant organ, of their target species. Alexis Carrel wrote in 1928 that '*tissues must originate from an animal susceptible to the disease of which the causal agent is being studied*' [11] and his view held sway for 20 years. Thus the idea that poliovirus would only grow in primate brain, a tissue not readily cultured *in vitro*, led to polio studies getting bogged down in costly and inconvenient animal work, entirely in monkeys until 1937 when Armstrong adapted type 2 poliovirus to mouse brain [12]. By then, however, as Enders wisely observed, '*it was becoming increasingly difficult to visualise the nervous system as the site of manufacture of the enormous quantities of virus...found in the faeces of many patients*' [13]. Enders, Robbins and Wellers' classic 1949 paper finally dispelled the idea of a narrow organotropism.

A second reason for delay was that throughout the 1930s most of the bacteriologists involved (the

designation 'virologist' did not exist) were growing animal viruses in fertile eggs or, in the case of vaccinia, still using the shaven skin of animals, either calves, sheep or rabbits. In some cases (influenza, yellow fever and pox viruses) egg culture was highly successful, but perhaps this was at the expense of a more timely adoption of *in vitro* cell culture. Though Carrel, a pioneer of tissue culture as applied to virology, had suggested as early as 1928 that '*a chick embryo crushed to a fine pulp is capable of producing as much smallpox vaccine as a calf*' [11], few could emulate his skill at that technique. Conservative forces meant that the vaccine was still being prepared on the flanks of animals right up to the global eradication of smallpox in the 1970s.

The difficulty of inoculating clinical specimens into cultures in the absence of antibiotic cover was a third reason why *in vitro* virus propagation was rarely attempted, or at any rate achieved, in the 1930s and 1940s. Microbial contamination was a major obstacle. As Wilson has recently commented: '*many researchers lacking Carrel's technical aptitude and financial resources met with regular failure when they attempted tissue culture*' [14]. A further constraint was the difficulty in obtaining the human embryonic or monkey tissue thought to be needed to propagate viruses such as polio.

The four factors that eventually energised the development of *in vitro* propagation of viruses were: the rediscovery of the dispersal of cells with trypsin as an alternative to the prolonged cutting up and mincing of tissue [15]; the development of defined cell growth and maintenance media; the availability of penicillin and streptomycin; and (conceptually) Enders and colleagues' demonstration that polioviruses were not exclusively neurotropic, but would grow in other tissues. Their refutation of the long held belief in neurotropism had immediate implications for the preparation of vaccines to meet the challenge of the post World War II epidemics of poliomyelitis, also for the expansion of viral diagnostics. When, for example, England and Wales' first public health virology laboratory opened in 1947 its work-book referred merely to the isolation of pox viruses on the chorio-allantoic membranes of eggs, the inoculation of specimens into mouse brains, and complement fixation tests using egg and brain-grown antigens [16]; but within a decade the use of monolayers of cells had become routine, as in several other national centres

worldwide. A diagnostic revolution had taken place, with monolayer cell culture at its heart [17].

VIROLOGISTS ADOPT THE MONOLAYER

For all that Enders and colleagues' paper was so influential, the effort required to propagate viruses in the suspended tissue fragments they had used in 1949, with the difficulties of observing the consequent effects on cell morphology, meant that that procedure quickly gave way to the preparation of cell monolayers. It was the readily maintained cell lines that could be grown as sterile monolayers on the inner surface of a test tube or medicine bottle and easily observed by light microscopy that made routine diagnostic virus culture feasible. Viruses that were host specific and organotropic in natural infection turned out to be less fastidious when inoculated onto monolayers of cells, presumably because the whole-host-dependent restrictive barriers (including immune defences) to cell entry were absent.

Virologists began eagerly to adopt those cell lines already found by biologists to be partially or completely immortal in *in vitro* culture. Cells of malignant tumours such as the HeLa and HEp 2 line proved particularly easy to maintain in long term culture, and increasing use was also made of cells such as adult monkey kidney [18,19]. These allowed the growth of a wide range of human viruses even though, after a few rounds of trypsinisation and re-seeding of the primary explant, their vitality fell away. Primary and secondary monkey kidney cells were particularly valued for the isolation from clinical specimens of polio and other enteroviruses, and certain respiratory viruses.

At this distance the scientific priorities for these crucial advances are not easy to assign, but it was George Gey, pathologist at the Johns Hopkins Medical School who, in February 1951, derived the HeLa line. His liberal distribution of this cell line to others' laboratories kick-started virus discovery. Gey began by collaborating with Syverton and Scherer of the University of Minnesota, and together the three of them realised the potential for distributing HeLa cells across the United States and beyond for diagnostic purposes. They could be grown on the internal surface of stoppered bottles and so readily dispatched as sterile monolayers to laboratories, both nationally and abroad.

Meanwhile, in Pasadena, California, Dulbecco inoculated monolayers of chick embryo cells grown on the surface of Petri dishes with serial dilutions of virus-bearing fluids. He over-layered the cells with agar so that plaques formed by the growth of individual virions could be visualised and enumerated [20]. This matched the way Koch, 70 years before, had used first gelatin and then agar to isolate and count bacterial colonies. Later Dulbecco and Vogt employed monolayers of trypsin-dispersed monkey kidney and other cells in the same way to study a wider range of viruses [21].

Cells with less continuous growth characteristics than HeLa cells and derived from adult and embryonic human and primate tissues, including amnion and embryonic lung and kidney [22,23], were soon incorporated into the available choice of monolayer cultures, and these played an important diagnostic role, widening the range of cell cultivable viruses. Local, national and international arrangements were made for the procurement, processing, supply and use of different cell monolayers for clinical diagnosis, and the known range of viruses capable of growing on monolayers expanded rapidly. In the United States, the National Foundation for Infantile Paralysis was a key supplier of monkey kidney tissue for the isolation of polio and other viruses. Monolayers of cells were also used for antigen preparation, serological testing, viral quantification and virus cloning [24].

The efflorescence of virus discovery that flowed from the introduction of monolayer cultures prompted an appreciation of the variety and numerical predominance of viral over other sorts of infections in the community, and so the growth in diagnostic demand was rapid. By the mid 1960s the study of infectious disease had been transformed by the incorporation of these virus isolation procedures: clinical virology had come of age.

CYTOPATHIC VIRUS EFFECTS

Many human viruses were found to have cytopathic effects (CPEs) on monkey kidney and other cell monolayers. These effects became visible by low power microscopy, usually between 24 and 120 hours later, and they were sometimes recognisably typical for a virus genus. The rapidity of the virus induced changes, whether in the pH of the medium or a focal or general shrinkage and destruction of cells, depended partly on the size

of the inoculum but mostly on the growth characteristics of the inoculated virus. Polioviruses, for example, rapidly destroyed cells, but some other viruses needed prolonged incubation of the monolayers, or one or more passages onto fresh monolayers, before a CPE was discernable. The appearance of giant cells and syncytia was sometimes a pointer to what the virus isolate might be, as was the ability to make the infected cells bind mammalian or avian red cells, or release a haemagglutinin detectable as a reaction between the culture fluid and the same range of red cells [25]. For more rapid diagnosis, cell sheets could be disrupted before the CPE was allowed to advance. The cell suspension was then clarified and the virus isolate pelleted from the supernatant by ultracentrifugation for diagnosis by electron microscopy. CPEs might be neutralised by the addition of convalescent or immune human or animal sera, allowing the development of identification tests for an infecting virus as well as, by reversal of the procedure, detection of serum antibodies to it [26]. With suitable controls, normally pre- and post-infection human or animal sera, a range of diagnostic possibilities opened up that involved only limited use of experimental animals and was faster and cheaper than alternative laboratory tests, in so far as any existed. The virologist could at last rival the bacteriologist in giving a timely diagnostic opinion. As a consequence new departments and chairs of virology began to be funded, at a rate and on a scale that it has not always been possible to sustain.

Monolayer cell culture also revealed previously unknown or at any rate uncharacterised human and animal viruses. Table 1, derived from Deinhardt and Henle [27], lists some early discoveries, and by 1967 the list had expanded several fold [7]. Intersecting pools of antisera were widely used to identify new virus species; these were sometimes 'orphan' viruses for which no disease correlate was known.

Naturally there were setbacks. A few important viruses such as hepatitis viruses resisted all attempts at propagation in cell monolayers. Some primary and continuous cell lines harboured unwanted viruses, both ones that caused a gross CPE such as 'foamy agent' and other cryptic simian viruses that could nevertheless interfere with diagnostic procedures [28]. Sometimes adventitious viruses were introduced with the

Table 1. Propagation of viruses in various cell monolayers (simplified from Deinhardt F and Henle G, 1957) CP = marked, cp = delayed cytopathic effect

	HeLa	KB	Intestine		Liver		Kidney	Conjunctiva	MCN	Monkey
			Chang*	407	Chang	407	Chang	Chang	(l cells)	Kidney
Herpes Simplex	CP	CP	CP	CP	CP	CP	CP	CP	CP	CP
Vaccinia	CP	CP	CP	CP	CP	CP	CP	CP	CP	
Vesicular Stomatitis	CP	CP	CP	CP	CP	CP	CP	CP	CP	
Polio I	CP	CP	CP	CP	CP	CP	CP	CP		CP
Polio II	CP	CP	CP	CP	CP	CP	CP	CP		CP
Polio III	CP	CP	CP	CP	CP	CP	CP	CP		CP
Coxsackie B1	CP	CP	CP	CP	CP	CP	CP		—	CP
Adeno 3	CP	CP	CP	CP	CP	CP	CP	CP		CP
Newcastle Disease	CP	CP	CP	CP	CP	CP	CP	CP	+ / -	CP
Mumps (PO)	CP	CP	CP	CP	CP	CP	CP	CP	+ / -	CP
Flu WSE	cp			cp		Cp			cp	cp

*Chang cells are embryonic cell lines.

animal serum used in cell growth media, and these infections were often occult, too. For example, calf serum was shown to be the source of a bovine polyoma virus found by electron microscopy to be a passenger in a continuous line of monkey kidney cells [29]. Many cell lines also became contaminated with mycoplasmata [30]. Nevertheless, virus isolation and neutralising serological tests were established as routine diagnostic investigations, and this greatly contributed to the demystification of viruses as agents of disease. Clinicians who saw febrile patients and epidemiologists who investigated outbreaks could often be told what the infecting virus was. Also the expansion of virus discovery sparked an interest in virus taxonomy which shows no sign of abating [31].

Nothing further happened in viral diagnostics to rival the introduction of monolayer culture until, in 1983, Kerry Mullis discovered how virus genomes could be amplified directly from specimens with suitable DNA primers. His polymerase chain reaction (PCR) has been of particular value in the study of hard-to-grow agents such as the hepatitis and gastroenteritis viruses, as well as the human immunodeficiency virus. Limitations on the availability of some tissues, disinclination to maintain a

full range of cell cultures, safety considerations and other more dubious rationalisations have since led to the view in some quarters that PCR and other gene amplification methods will supersede monolayer culture; but this notion is misguided. In particular, cell culture allows genomically uncertain viruses to be discovered, propagated and characterised. For instance, while it was possible to identify the SARS coronavirus using PCR, the virus also turned out to grow with alarming ease in Vero and other cells. Hybrid approaches involving the initial inoculation of monolayers followed after a few hours by PCR have recently been described. These may heighten the sensitivity and increase the speed of viral diagnosis.

CONCLUSION

The legacy of Rous, Enders, Gey, Syverton and Scherer, Dulbecco and others whose discoveries have been so widely drawn upon, lives on (Table 2). The published record of their work stands and their names still resonate in virological circles. They described cell culture procedures which several generations of laboratory technologists have since used, lately often in ignorance of

Table 2. Some milestones in viral diagnosis by tissue/cell culture

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- Inoculation of tissue fragments suspended in plasma enriched medium (Maitlands, 1928)
 - Inoculation of tissue fragments suspended in Tyrode's medium (Li and Rivers, 1931)
 - Development of defined media (various authors, 1940s)
 - Use of penicillin and streptomycin (1949 onwards)
 - Inoculation of HeLa monolayers (Gey, Syverton and Sherer 1953)
 - Inoculation of monkey kidney monolayers (Youngner 1954)
 - Plaquing of viruses on monolayers under agar (Dulbecco and Vogt 1954)
 - Haemadsorption to detect virus growth (Vogel and Shelokov 1957)
-

their derivation. Anyone tempted to belittle these pioneers' contributions should read the descriptions of the painstaking tissue culture procedures that monolayer cell culture superseded, not to mention the animal work of a yet earlier era which it would now be very contentious to have to rely on. Monolayer cell culture established virology as an independent academic discipline, and while Enders and colleagues' classic paper was only one step on the way it had a lasting impact on diagnostic medicine. It is a sign of the fundamental importance of the technique of cell culture of viruses that it is now taken for granted!

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