

# *A Brief History of Human Diploid Cell Strains*

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The Pontifical Academy for Life published a response about the moral legitimacy of immunizing children with vaccines manufactured using cell strains derived from aborted human fetuses. In order to fully appreciate the level of cooperation involved among different agents, it is important to review the history of the development of these cell strains: “The need to articulate a moral reflection on the matter in question arises mainly from the connection which exists between the vaccines mentioned above and the procured abortions from which biological material necessary for their preparation was obtained.”<sup>1</sup>

Human diploid cell strains (HDCSs) are batches of cells that are currently used for different purposes, including culturing viruses for the manufacturing of vaccines. HDCS-derived human vaccines have been licensed worldwide for polio IVP and OVP, rabies, rubella, measles, varicella-zoster, mumps, and hepatitis A. Current vaccines contain extremely small traces of the original fetal DNA, while the cell strains contain the complete fetal chromosomal set. The choice of HDCS was made among several based on their susceptibility to many human viruses, their good characterization, the enormous number of cells obtained from one original culture, their long

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<sup>1</sup> Pontifical Academy for Life, “Moral Reflections on Vaccines Prepared from Cells Derived from Aborted Human Fetuses” (June 5, 2005), <http://www.academiavita.org/template.jsp?sez=Documenti&pag=testo/vacc/vacc&lang=English>; reprinted in this issue of the *Quarterly* on pp. 541–549.

storage potential, the low cost of cell procurement, an excellent record of safety, and the very low risk of latent virus on the cells themselves.<sup>2</sup>

Even though there are many cell strains in use in research, the most well known are WI-38 and MRC-5. These two cell strains come from two deliberately aborted fetuses. But as the evidence shows, there were more abortions involved to achieve the technical expertise needed for development of these cell strains. In addition, other cell strains have been developed for vaccine manufacturing and other purposes. Because of its relevance to this discussion, I will also review the history of the virus strain RA 27/3, as it is the source of the only rubella vaccine available in North America and in fact, most of the world. Finally, as my intention is to capture the real meaning of the evidence, I will quote from the actual sources and personal communications to try to respect the original meaning.

### Human Diploid Cell Strains

The Wistar Institute is a scientific institute located on the campus of the University of Pennsylvania in Philadelphia, specializing in the fields of immunology and cell biology. Working for the Institute in 1961, Dr. Leonard Hayflick first published a paper describing twenty-five HDCS: WI-1 through WI-25 (Wistar Institute fetal samples nos. 1–25). These cell strains were derived from the lung, skin, muscle, kidney, heart, thyroid, thymus and liver of nineteen separate, electively-aborted fetuses. The purpose of choosing different organs was to test difference in tissue characteristics. His research included also testing these cell strains' susceptibility for different viruses. He stated in this paper that

the isolation and characterization of HDCS from fetal tissue make this type of cell available as a substrate for the production of live virus vaccines. Other than the economical advantages, such strains ... make the consideration of their use in the production of human virus vaccine a distinct possibility.<sup>3</sup>

Abortion was illegal in the United States at that time, so fetal tissue was provided by Dr. Sven Gard of the Karolinska Institute Medical School in Stockholm, Sweden.<sup>4</sup> Dr. Erling Norrby, who later served as chairman of the department of virology and dean of the medical faculty at the Karolinska Institute, was a graduate student there during this period. He dissected many of the aborted fetuses:

My predecessor as professor of virology at the Karolinska Institute in Stockholm, Sven Gard, spent a sabbatical year at the Wistar Institute in 1959,

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<sup>2</sup>M. A. Fletcher, L. Hessel, and S. A. Plotkin, "Human Diploid Cell Strains (HDCS) Viral Vaccines," *Developments in Biological Standardization* 93 (1998): 97–107; L. Hayflick, "History of Cell Substrates Used for Human Biologicals," *Developments in Biological Standardization* 70 (1989): 11–26.

<sup>3</sup>L. Hayflick and P. S. Moorhead, "The Serial Cultivation of Human Diploid Cell Strains," *Experimental Cell Research* 25.3 (December 1961): 618.

<sup>4</sup>E. Norrby, "Listen to the Music: The Life of Hilary Koprowski (review)," *Perspectives in Biology and Medicine* 44.2 (Spring 2001): 304; Fletcher, Hessel, and Plotkin, "Human Diploid Cell Strains," 97–98.

two years after the institution had been taken over by the dynamic Koprowski. One of my duties as a young student in the laboratory in Stockholm was to dissect human fetuses from legal abortions and send organs to the Wistar Institute. Such material was the source of many important studies of cell lines at the Institute, such as Leonard Hayflick's study of WI-38 cells.<sup>5</sup>

Hayflick and his collaborators (including Anthony Girardi from the Merck Institute for Therapeutic Research) started working with these cell strains to develop viral vaccines: a poliovirus vaccine was developed in the WI-1 cell strain in 1962. By this time, fifty HDCSs had been made. Finally, after these improvements in the technique, Hayflick published his reports of the development of WI-38.<sup>6</sup> WI-38 was obtained from a three-month-old female fetus:

This fetus was chosen by Dr. Sven Gard, specifically for this purpose. Both parents are known, and unfortunately for the story, they are married to each other, still alive and well, and living in Stockholm, presumably. The abortion was done because they felt they had too many children. There were no familial diseases in the history of either parent, and no history of cancer specifically in the families.<sup>7</sup>

This report also mentions two additional cell strains: WI-26 from a male fetus (lung) and WI-44 from a female fetus (lung). Both fetuses were about three-months' gestation as well.<sup>8</sup>

An article co-authored by Gard and colleagues at the Wistar Institute stated, in reference to Hayflick's cell strains, that

a human diploid cell strain derived from a fetal lung tissue was employed instead of monkey-kidney cells for the preparation of the attenuated poliovirus vaccine utilized in our study. The cell strain, cultivated especially for the production of virus vaccines, retains relatively constant morphology and chromosomal characteristics ... and it is believed to be free of all known adventitious agents. The expectation is that cells originating from a single fragment of tissue, passages of which are stored and cultivated at will, could be used in place of monkey cells ... to make large quantities of vaccine.<sup>9</sup>

On an interesting note, Hayflick was concerned about the continued capture of wild monkeys and their existence as species and saw HDCS as a solution to this

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<sup>5</sup> Norrby, "Listen to the Music," 304.

<sup>6</sup> L. Hayflick, "The Limited In Vitro Lifetime of Human Diploid Cell Strains," *Experimental Cell Research* 37 (March 1965): 614–636; L. Hayflick et al., "Preparation of Poliovirus Vaccines in a Human Fetal Diploid Cell Strain," *American Journal of Hygiene* 75 (March 1962): 240–258.

<sup>7</sup> "Gamma Globulin Prophylaxis; Inactivated Rubella Virus; Production and Biologics Control of Live Attenuated Rubella Virus Vaccines" [no author given], *American Journal of Diseases of Children* 118.2 (August 1969): 377–278.

<sup>8</sup> Hayflick et al., "Preparation of Poliovirus Vaccines," 240, 244, 254.

<sup>9</sup> J. S. Pagano et al., "The Response and the Lack of Spread in Swedish School Children Given an Attenuated Poliovirus Vaccine Prepared in a Human Diploid Cell Strain," *American Journal of Hygiene* 79 (January 1964): 74–75.

problem. A previous ethical version of these vaccines was developed from the kidney cells of the African green monkeys, an endangered species.<sup>10</sup> Also, Hayflick himself became a vaccine developer for a polio vaccine and fought and won the legal right to hold a patent and profit from WI-38.<sup>11</sup> Finally, Hayflick was one of the co-signers of a letter sent to President Bush in 2001 to support the destruction of human embryos that occurs in embryonic stem cell research:

For the past thirty-five years many of the common human virus vaccines—such as measles, rubella, hepatitis A, rabies and poliovirus—have been produced in cells derived from a human fetus to the benefit of tens of millions of Americans. Thus precedent has been established for the use of fetal tissue that would otherwise be discarded.<sup>12</sup>

He is on the scientific advisory board of Advanced Cell Technology, the private company that claimed to have cloned the first human embryo in 2002.

Dr. J. P. Jacobs published the development of the cell strain MRC-5 (Medical Research Council strain no. 5) in 1970. He replicated Hayflick's work with the purpose of creating cells strains for the production of vaccines:

The stability and integrity of the human foetal cell strain WI-38 ... explain the value of such material for the isolation of viruses and in the development of vaccines. We have developed another strain of cells, also derived from fetal lung tissue, taken from a fourteen-week male fetus removed for psychiatric reasons from a twenty-seven-year-old woman with a genetically normal family history and no sign of neoplastic disease both at abortion and for at least three years afterwards.<sup>13</sup>

There is the possibility that there may have been previous abortions performed to create MRC-5. In fact, Jacobs reported creating a second cell strain, MRC-9, by the use of a different aborted fetus:

the cells were derived from the lungs of a female fetus in 1974, whose gestational age was about fifteen weeks. The fetus was of normal development and was delivered of a fourteen-year-old mother whose pregnancy was terminated by therapeutic abortion because she was unmarried. The medical history of the mother and her family indicated nothing abnormal according to information given by the gynecologist who performed the operation. The lungs were dissected from the fetus immediately following the abortion...<sup>14</sup>

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<sup>10</sup> L. Hayflick, "The Choice of the Cell Substrate for Human Virus Vaccine Production," *Laboratory Practice* 19.1 (January 1970): 59.

<sup>11</sup> L. Hayflick, "History of Cell Substrates Used for Human Biologicals," *Developments in Biological Standardization* 70 (1989): 15.

<sup>12</sup> K. J. Arrow et al., "Nobel Laureates' Letter to President Bush," *Washington Post*, February 22, 2001, A02.

<sup>13</sup> J. P. Jacobs, C. M. Jones, and J. P. Baille, "Characteristics of a Human Diploid Cell Designated MRC-5," *Nature* 227.5254 (July 11, 1970): 168.

<sup>14</sup> J. P. Jacobs, A. J. Garrett, and R. Merton, "Characteristics of a Serially Propagated Human Diploid Cell Designated MRC-9," *Journal of Biological Standardization* 7.2 (April 1979): 114.

Newer HDCSs continued to be made as back-ups for the current cell strains. Among the most common ones are IMR-90, cell strain 293, and PER C6.<sup>15</sup> In short, IMR-90 was established from a sixteen-week-old human fetus on July 7, 1975, from a therapeutic abortion performed on a thirty-eight-year-old white mother of six.<sup>16</sup> Cell strain 293 was made from human embryonic kidney cells from an aborted fetus in 1972, and cell strain PER C6 from human embryonic retina cells from an abortion in 1985. The main researcher was Dr. A. J. van der Eb at Leiden University in Holland. Van der Eb dissected the fetuses himself, which were healthy. PER C6 came from an eighteen-week-old aborted fetus because “the woman wanted to get rid of the fetus and the father was unknown.” Van der Eb stated that “PER C6 was made just for the pharmaceutical manufacturing of adenovirus vectors.” He also added, “I realize that this sounds a bit commercial, but PER C6 was made for that particular purpose.” Cell strain 293 was made for “basic research.”<sup>17</sup> At least fifty companies have licensed PER C6, including Merck, the sole manufacturer of the only rubella vaccine available in North America.<sup>18</sup>

### **The Origin of Rubella Virus RA 27/3**

Currently, the virus strain (RA 27/3) found in the rubella vaccine most commonly used around the world was developed by Dr. Stanley Plotkin and colleagues at the Wistar Institute.<sup>19</sup> The RA 27/3 (rubella abortus, twenty-seventh fetus, third tissue extract) virus strain was obtained from a female human fetus in a series of twenty-seven abortions in the United States: “Explant cultures were made of the dissected organs of a particular fetus aborted because of rubella, the twenty-seventh in our series of fetuses aborted during the 1964 epidemic.”<sup>20</sup> “This fetus was from a twenty-five-year-old mother exposed to rubella eight weeks after her last menstrual period. . . . The fetus was surgically aborted seventeen days after maternal illness and dissected immediately. . . . It was then grown on WI-38.”<sup>21</sup>

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<sup>15</sup> W. W. Nichols et al., “Characterization of a New Human Diploid Cell Strain, IMR-90,” *Science* 196.4285 (April 1, 1977): 60; FDA Vaccines and Related Biological Products Advisory Committee, transcript of meeting May 16, 2001, “Session on Designer Cell Substrate,” [http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3750t1\\_01.pdf](http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3750t1_01.pdf).

<sup>16</sup> Nichols et al., “Characterization of a New Human Diploid Cell Strain,” 60.

<sup>17</sup> Alex J. van der Eb, in “Session on Designer Cell Substrates,” FDA meeting transcript, May 16, 2001.

<sup>18</sup> L. Xie et al., “Large-Scale Propagation of a Replication-Defective Adenovirus Vector in Stirred-Tank Bioreactor PER.C6 Cell Culture under Sparging Conditions,” *Biotechnology and Bioengineering* 83.1 (July 5, 2003): 45.

<sup>19</sup> S. A. Plotkin, D. Cornfeld, and T.H. Ingalls, “Studies of Immunization with Living Rubella Virus: Trials in Children with a Strain Cultured from an Aborted Fetus,” *American Journal of Diseases of Children* 110.4 (October 1965): 381–382.

<sup>20</sup> S. A. Plotkin et al., “Attenuation of RA 27-3 Rubella Virus in WI-38 Human Diploid Cells,” *American Journal of Disabilities of Children* 118.2 (August 1969): 178.

<sup>21</sup> Plotkin, Cornfeld, and Ingalls, “Studies of Immunization with Living Rubella Virus,” 381–382.

The new vaccine was tested on children at a Roman Catholic orphanage in Philadelphia. It is documented that there were other effective virus strains already made at that time which had been obtained from other non-abortion-related methods.<sup>22</sup> Nevertheless, the researchers seem to have chosen RA 27/3 because of its lack of contaminants, immunogenicity, low side effects, and enormous cell growth. Also, RA 27/3 was further cultured with the cell strain WI-38.

Additionally, six months after publishing this research, Plotkin and colleagues published an article documenting forty, not twenty-seven, abortions:

Out of the forty rubella fetuses cultured, cell strains were derived from thirty-four; in most cases they originated from pieces of skin and muscle obtained at curettage ... rubella virus was isolated from the supernatant culture medium of cell strains derived from eighteen fetuses; sixteen fetuses yielded cell strains which were rubella negative.<sup>23</sup>

The RA 27 fetus was not the first fetus in the series to be positive for rubella virus. It is not clear why they continued with the series.

Later, Drs. J. Hoskins and Plotkin tested the action of the RA 27/3 virus on different systems of human embryonic cell cultures. Additional cell strains were made from more fetuses originating in both elective abortions (twenty-one) and miscarriages (seven):<sup>24</sup>

Two groups of human fetuses, eight to twenty weeks, were used for the initiation of diploid cell strains. The first group consisted of normal embryos obtained by hysterotomy and flown from Scandinavia... The second group represented spontaneous abortions obtained from the gynecologic service of the Philadelphia General Hospital.<sup>25</sup>

From the records, it also seems that both sources of cell strains yielded similar efficacy:

Cell strains derived from twenty-nine fetuses were examined. Twenty one of these originated from surgical abortions, while seven came from spontaneously aborted fetuses. One cell strain was of uncertain origin. At the start of these studies, most importance was attached to HDCS derived from the surgically aborted fetuses since these could be presumed to be normal. In fact, no differences in any of the parameters studied could be found between the two groups of fetuses, and no distinction will henceforth be made between them.<sup>26</sup>

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<sup>22</sup> F. T. Perkins, "Licensed Vaccines," *Reviews of Infectious Diseases* 7 (March–April 1985), Suppl 1: S73–S76.

<sup>23</sup> T. H. Chang et al., "Chromosome Studies of Human Cells Infected in Utero and In Vitro with Rubella Virus," *Proceedings of the Society for Experimental Biology and Medicine* 122.1 (May 1966): 237–238.

<sup>24</sup> J. M. Hoskins and S. A. Plotkin, "Behaviour of Rubella Virus in Human Diploid Cell Strains. I. Growth of Virus," *Archiv fur die Gesamte Virusforschung* 21.3 (1967): 285; J. M. Hoskins and S. A. Plotkin, "Behaviour of Rubella Virus in Human Diploid Cell Strains. II. Studies of Infected Cells," *Archiv fur die Gesamte Virusforschung* 21.3 (1967): 297.

<sup>25</sup> Hoskins and Plotkin, "Behaviour of Rubella Virus I," 285.

<sup>26</sup> Hoskins and Plotkin, "Behaviour of Rubella Virus II," 297.

(Please note the arithmetic error, as twenty-one and seven do not add up to twenty-nine fetuses. It could have been one more aborted fetus or one extra miscarriage).

Plotkin later developed experimental polio, varicella, and cytomegalovirus vaccines. He is now employed at Sanofi Pasteur, a vaccine manufacturer. He believes that his rubella vaccine has helped to prevent many abortions: ““I have no doubt that rubella vaccination has prevented thousands and thousands of abortions,”” he said. ‘From strictly an arithmetical assessment, the good done by the vaccine—if you are opposed to abortion—is infinitely greater than any possible harm.’”<sup>27</sup>

### **The Manufacturers**

At this point, it is important to mention that pharmaceutical companies in both Europe and North America quickly became involved in the use of HDCSs.<sup>28</sup> The World Health Organization in joint effort with the Wistar Institute funded meetings and training sessions with individuals interested in learning about HDCSs during the 1960s.<sup>29</sup>

Given the fact that the research was public knowledge, it is impossible that the companies were unaware of the ethical predicament. To the researchers’ credit, they never hid the real source of the cells, as the titles of their articles confirm.<sup>30</sup> As stated in the written evidence, at least one collaborator in the research was working for a pharmaceutical company at the time the research was being done. Besides, the minimum requirements of safety standards dictate that a manufacturing pharmaceutical company know in detail the source of its raw material. The question has been posed whether this is like benefiting from the use of an organ from an executed persons or from unethical Nazi research.<sup>31</sup>

### **The Abortions**

As I am also preparing an article for a Canadian medical journal on immunization, refusal, safety, and informed consent but not necessarily on the moral point of view, I needed to be able to assess the vaccines’ track of safety. In order to do this, it was necessary to trace back to the original abortions to ensure that there were no foreign dangerous contaminants. I thus emailed Dr. Norrby about this problem. Dr. Norrby stated that the cell strains were safe, since the tissue was collected in a very sterile manner:

When we collected the organs this was done immediately after the legal abortion. We were on duty to immediately perform the sampling and to arrange for

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<sup>27</sup> D. Brown, “Rubella Virus Eliminated in the United States,” *Washington Post*, March 21, 2005, A07.

<sup>28</sup> Fletcher, Hessel, and Plotkin, “Human Diploid Cell Strains,” 97–98.

<sup>29</sup> Hayflick, “History of Cell Substrates,” 15.

<sup>30</sup> Plotkin, Cornfield, and Ingalls, “Studies of Immunization with Living Rubella Virus,” 381–382.

<sup>31</sup> R. K. Zimmerman, “Ethical Analyses of Vaccines Grown in Human Cell Strains Derived from Abortion: Arguments and Internet Search,” *Vaccine* 22.31–32 (October 22, 2004): 4238–4244.

an as rapid transport as possible over the Atlantic Ocean. The fetal material arrived by car from the nearby hospital to our laboratory enwrapped in a green surgical cloth. Maximal sterility was critical to allow an outgrowth of fetal cells without any contamination after the transport under cold conditions to the Wistar Institute.<sup>32</sup>

Whether there was any coercion in the abortions in order to procure these cell strains is unknown. We will also probably never know whether the mothers were actually aware that their abortions may have been used for the creation of cell strains, given what Dr. Norrby states regarding informed consent:

Remember that at the time in the early 1960s when organs from aborted fetuses were collected and sent to the Wistar Institute no one had as yet invented the concept of informed consent. I am absolutely convinced that there is no remaining documentation about the fetuses used from the Department of Virus Research of the Karolinska Institute at the time. I was the head of this department between 1972 and 1997. Thus in case there is no documentation that allows identification of fetal samples at the Wistar Institute, there is no way of tracing them. I do in fact remember the time well, because we as graduate students made the dissections collecting organs.<sup>33</sup>

### Conclusion

There is clear evidence that research around the development of the RA 27/3 rubella vaccine included the performance and coordination of at least eighty abortions, including the two individual abortions for the creation of WI-38 and RA 27/3. Development of MRC-5 used one abortion, but there is a strong indication that more abortions occurred. Evidence also seems to indicate that there was intention in the act of utilizing abortions for the creation of cell strains, most likely because the tissue source ensures an absence of contamination and a high growth titer. There have been other abortions as a result of the need to create more cell strains for use in vaccine development.<sup>34</sup> Pharmaceutical companies are actively involved in this research and

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<sup>32</sup> E. Norrby, e-mail response to a message from R. Leiva on January 23, 2006. Dr. Leiva had asked: "You mention that the step you were involved in (dissection of the fetal tissue) was done under sterile conditions. What about the steps of the procedure prior to that? Do you know anything about the conditions between the therapeutic abortions and the dissections? Were they both happening one after the other in the same facility and laboratory standards?"

<sup>33</sup> E. Norrby, e-mail response to a message from R. Leiva on January 20, 2006. Dr. Leiva had asked: "(1) Was the reason for the pregnancies' termination medical or socio-therapeutic? (i.e., were diseases in the fetuses the reasons for the terminations?) (2) Was there good documentation regarding the health of parents of the fetuses? If so, where this can be obtained? (3) How were particular fetuses chosen? (Were there any medical reasons for choosing a particular fetus as Dr. Gard says in reference 2, or did the parents have any input in the choice. And (4) How was the termination–dissection–set-up organized to decrease the risk of introducing any kind of contaminants?"

<sup>34</sup> M. G. Pau et al., "The Human Cell Line PER.C6 Provides a New Manufacturing System for the Production of Influenza Vaccines," *Vaccine* 19.17–19 (March 21, 2001): 2716–2721.



new vaccines are being made with unethical cell strains.<sup>35</sup> There are alternative ethical viral vaccines already made with modern cell substrates: Cell lines such as mammalian cells like Vero monkey cells and Chinese hamster ovary cells (e.g. some Polio IVP).<sup>36</sup> Alternatively, producing vaccines with antigens using recombinant DNA technology is another option (e.g.: hepatitis B).<sup>37</sup> Efforts should be made to encourage research on these and other novel ethical sources.

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<sup>35</sup> M. N. Oxman et al. for the Shingles Prevention Study, "A Vaccine to Prevent Herpes Zoster and Postherpetic Neuralgia in Older Adults," *New England Journal of Medicine* 352.22 (June 2, 2005): 2271–2284.

<sup>36</sup> L. Hayflick, "History of Cell Substrates," 24.

<sup>37</sup> D. B. Huang, J. J. Wu, and S. K. Tying, "A Review of Licensed Viral Vaccines, Some of their Safety Concerns, and the Advances in the Development of Investigational Viral Vaccines," *Journal of Infection* 49.3 (October 2004): 179–209.