Enders Measles Paper (1954)

John Franklin Enders 1954 paper is considered the definitive proof for the discovery of a measles “virus.” This evidence was presented as the “isolation” of measles and served as the basis for which the vaccine was built upon. It also laid the groundwork for the use of cell culture experiments as a way to cultivate “viruses” as well as the resulting claims that the indirect evidence known as cytopathogenic effects (CPE) can act as a surrogate measure for “viruses” being present in the toxic cell culture soup. Reading the paper and Enders conclusions, however, tells a completely different story than the isolation of a “virus.” Highlights from his paper below:

Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles.

“Numerous attempts have been made in the past to propagate the agent of measles in lower animals, in chick embryos and in tissue cultures(1-3). **The results of different investigators were often at variance or directly contradictory.** It has been made reasonably clear, however, **that monkeys, especially M. mulatta, are moderately susceptible to experimental inoculation** (3). Furthermore the researches of Rake, Shaffer and their collaborators have **provided evidence suggesting that the agent which passed through bacteria-retaining filters could be maintained indefinitely in serial passages in the developing chick embryo** (4,s). These workers (5) also confirmed the earlier observations of Plotz(6) who **apparently had succeeded** in growing the agent in a modified suspended cell culture of chick embryonic tissues. Egg passage in the hands of Shaffer and his coworkers(7,8) **regularly appeared to alter the pathogenicity of the agent for man as indicated by the development of a mild and much modified disease following the inoculation of egg adapted materials into susceptible children**. In certain cases this modified disease

seemed to be followed by resistance to measles **as indicated by the results of subsequent natural or artificial exposure to the virulent form of the agent(9).** Since 1943 when the last of the communications by Rake and his collaborators appeared, no important progress has been made in the study of the etiology of measles. **This fact may in large part be attributed to the lack of a convenient laboratory method for the demonstration of the presence of the agent which induced no recognizable changes in eggs or cultures of chick tissues.** More-over, **repeated attempts by Shaffer(10) to demonstrate a serologic reaction, such as complement fixation, using materials from the infected chick embryo failed**. Accordingly, the only available technics have consisted in the inoculation of man or the monkey. The former is obviously impractical as routine and the latter tedious, expensive and frequently inconclusive because of variation in individual susceptibility.

With these considerations in mind we have recently attempted to cultivate the agent of measles in cultures of human and monkey cells employing procedures applied successfully to the propagation of the poliomyelitis viruses (11-13). 4 In blood and throat washings of typical cases of measles agents have been demonstrated that **can be maintained in serial passage in tissue cultures and which induce distinctive cytopathic changes in renal epithelial cells**. A certain amount of evidence has been accumulated indicating that antibodies specific for these agents develop during the course of the disease. It is our purpose to describe here these observations in a preliminary manner. Additional evidence for the relationship of these agents to measles will be sought in future investigations.

Materials and methods. Collection of specimens. Throat washings, venous blood and feces were obtained from 7 patients as early as possible after a clinical diagnosis of measles was established. In 5 instances the time at which specimens were collected in relation to the onset of exanthem is given in the case histories described below or in Table I.

When capable, **patients were asked to gargle with 10-15 ml of sterile neutralized fat-free milk. Certain specimens from the throats of younger children were obtained by cotton swab previously moistened in milk**. After swabbing the throat **the swab was immersed in 2 ml of milk. Penicillin, 100 u/ml, and streptomycin, 50 mg/ml. were added to all throat specimens** which were then centrifuged at 5450 rpm for about one hour. Supernatant fluid and **sediment resuspended in a small volume of milk** were used as separate inocula in different experiments in amounts varying from 0.5 ml to 3.0 ml. **About 10 ml of blood immediately after withdrawal were placed in tubes containing 2 ml of 0.05% solution of heparin**. As inocula for tissue cultures amounts varying from 0.5 ml to 2.0 ml of the whole blood were employed. **After addition of antibiotics as described above 10% fecal suspensions were prepared by grinding the material in bovine amniotic fluid medium**. The suspensions were then centrifuged at 5450 rpm for about one hour and the supernatant fluids used as inocula, in amounts varying from 0.1 ml to 3 ml. All specimens were refrigerated in water and ice or maintained in the cold at about 5°C from the time of collection until they were added to the cultures. The maximum time that lapsed between collection of specimens and inoculation was 35 hours."

"Tissue culture technics. In the initial isolation attempts roller tube cultures (1 1 12) of human kidney, human embryonic lung, human embryonic intestine, human uterus and rhesus monkey testis were employed. Subsequent passages of the agents isolated were later attempted in human kidney, human embryonic skin and muscle, human foreskin, human uterus, rhesus monkey kidney and embryonic chick tissue. Stationary cultures prepared according to the technic of Youngner(13) **with trypsinized human and rhesus monkey kidney were later employed for isolation of agents and their passage. The culture medium consisted of bovine amniotic fluid (go%), beef embryo extract (50/0), horse serum (5%), antibiotics, and phenol red as an indicator of**

cell metabolism (1 2). Soybean trypsin inhibitor was added to this medium unless it was used forthe cultivation of human and monkey kidney (11). Fluids were usually changed at intervals of 4-5 days. For histological examination the cell growth after fixation in 10% formalin was embedded in collodion, dehydrated and stained with hematoxylin and eosin."

"Manner of passage in tissue culture. **Serial passage of the various strains (Table I) was accomplished as routine by removal of the culture medium between the 4th and the 16th day after inoculation and immediate transfer of 0.1 ml to each of a number of fresh cultures.** Successful passage of the agent with fluids that had been previously centrifuged at 2500 rpm to remove cellular elements has also been
repeatedly demonstrated. Larger inocula (up to 1.0 ml) were often used during the initial experiments before the resistance of the agent to storage at various temperatures had been determined."

"Experimental. Cytopathic changes induced by agents isolated from cases of measles. The first of 8 agents obtained from blood or throat washings of measles cases and exhibiting comparable properties was isolated in cultures of human kidney tissue following addition of the blood of Case 3. In each of the 3 cultures that were inoculated cytopathic changes were observed on the 7th day. Since **these changes presented a characteristic appearance not heretofore associated definitely with a virus** they have provided the means for the further investigation of this agent as well as others that have been recently isolated."

"Examination of stained materials also revealed significant changes within the nuclei of the giant cells that were not visible in fresh preparations. These consisted in a redistribution of the chromatin which ultimately assumed a marginal position where it formed a dense ring or crescent that stairied intensely with the basic dye. Concomitantly the central portion of the nucleus came to be occupied by an apparently homogeneous substance, acidophilic in character, that approximated closely

to the chromatin ring. Since in these and other preparations that have been examined subsequently no clear unstained zone has been observed between the chromatin and this acidophilic mass, **it cannot be asserted that the latter represents an intranuclear inclusion body of the type characteristically associated with viral infections**. Nevertheless, as far as can now be determined, its presence along with the margination of the chromatin affords a useful criterion of infection for the agents under study. **It should be emphasized, however, that the changes as just depicted are encountered in cultures that have been incubated for relatively prolonged periods (e.g. 14-21 days). When the interval between inoculation of the agent and examination of the stained cells (e.g. 4 days) is shorter, margination of the chromatin may be incomplete or inapparent** and the acidophilic substance may only be seen in small rounded masses distributed here and there amid nuclear materials that approximate the normal arrangement."

"B) Cytopathogenic range. **Monkey kidney is the only other tissue employed that has yielded a growth of cells in which the characteristic changes described above have been definitely observed following inoculation of virus.** In cultures consisting largely of monkey renal epithelial cells as prepared by Youngner's modification of Dulbecco's technic (13) cytopathic changes have been regularly observed which resemble closely those produced by these agents in human renal cells as seen in both fresh and stained preparations. These effects followed the addition of blood or throat washings from cases of measles as well as infected tissue culture fluids derived from previous passages. Monkey kidney cultures may, therefore, be applied to the study of these agents in the same manner as cultures of human kidney. **In so doing, however, it must be borne in mind that cytopathic effects which superficially resemble those resulting from infection by the measles agents may possibly be induced by other viral agents present in the monkey kidney tissue (cf. last**

paragraph under G) or by unknown factors. In a few cultures of human prepucial tissue inoculated with one of the measles agents changes resembling those seen in renal cells were noted in the epithelial outgrowth about certain fragments. Additional observations, however, will be required before it can be confidently asserted that dermal epithelial cells are specifically attacked by these viruses. In a single experiment no cytopathic manifestations were seen during a period of 31 days following inoculation of infected tissue culture fluid into cultures of human embryonic skin and muscle, human uterine tissue or embryonic chick tissue. Tests for the presence of complement fixing antigen in the fluids removed from the cultures on the 31st day were negative. These serologic results suggest that growth of the virus did not occur, since, as will be shown subsequently, the antigen appears to develop regularly after several days in cultures of renal tissue infected with the virus."

"Other agents isolated during this study. **Two agents have been isolated while the present work was in progress that appear unrelated to those we have just described**. The first was recovered from the throat washings of a typical case of measles occurring in the boys' school. Its wide cytopathogenic range, the character of the cytopathic changes induced and the fact that its infectivity for tissue cultures was neutralized by herpes simplex immune rabbit serum served to define its nature. A second agent was obtained from an uninoculated culture of monkey kidney cells. **The cytopathic changes it induced in the unstained preparations could not be distinguished with confidence from the viruses isolated from measles.** But, when the cells from infected cultures were fixed and stained, their effect could be easily distinguished since the internuclear changes typical of the measles agents were not observed. Moreover, as we have already indicated, fluids from cultures infected with the agent failed to fix complement in the presence of convalescent measles serum. **Obviously the possibility of encountering such agents in studies with measles should be constantly kept in mind.**

Discussion. Of the numerous experiments that have been reported in the past describing the successful isolation of the etiologic agent of measles only those in which monkeys were employed as the experimental animal have been consistently confirmed by other workers. **Great caution should therefore be exercised in the interpretation of any new claims that the virus has been propagated in other hosts or systems. Accordingly, the results that are summarized here must be subjected to the most critical analysis.**

The following facts tend to **support the hypothesis that the viruses we have described are responsible for the disease.** Experimentally transmissible agents exhibiting a similar and characteristic cytopathogenic effect in cultures of human or simian epithelial cells have been isolated from either the blood or throat washings derived from 5 of 7 typical cases of measles during the early acute phase. An agent was demonstrated in the blood of 4 of the 5 cases from which specimens were obtained and examined by the tissue culture method. These findings would seem to be of especial significance since it is unlikely that viruses unrelated to measles would be regularly present in the circulating blood of these individuals some of whom were geographically widely separated.

The pathologic changes induced by the agents in epithelial cells in tissue culture resemble, **at least superficially**, those found in certain tissues during the acute stage of measles. **While there is no ground for concluding that the factors in vivo are the same as those which underlie the formation of giant cells and the nuclear disturbances in vitro,** the appearance of these phenomena in cultured cells is consistent with the properties **that a priori might be associated with the virus of measles.**

The emergence of antibodies during the course of the disease capable of suppressing the cytopathogenic effect and of fixing complement in the presence of infected tissue culture fluids affords **further evidence for the close association** of the agents with measles. Obviously additional data to be derived

from tests with sera from a large number of cases of measles as well as other infectious diseases, especially the common exanthemata, **are desirable in order to eliminate any remaining doubt concerning the specificity of these serologic reactions**. The accumulation of such data is now in progress.

Although we have thus already **obtained considerable indirect evidence** supporting the etiologic role of this group of agents in measles, 2 experiments essential in the establishment of this relationship remain to be carried out. **These will consist in the production of measles in the monkey and in man with tissue culture materials after a number of passages in vitro sufficient to eliminate any virus introduced in the original inoculum. The recovery of the virus from the experimental disease in these hosts should then be accomplished**.

Conclusion. The findings just summarized **support the presumption that this group of agents is composed of representatives of the viral species responsible for measles.**“

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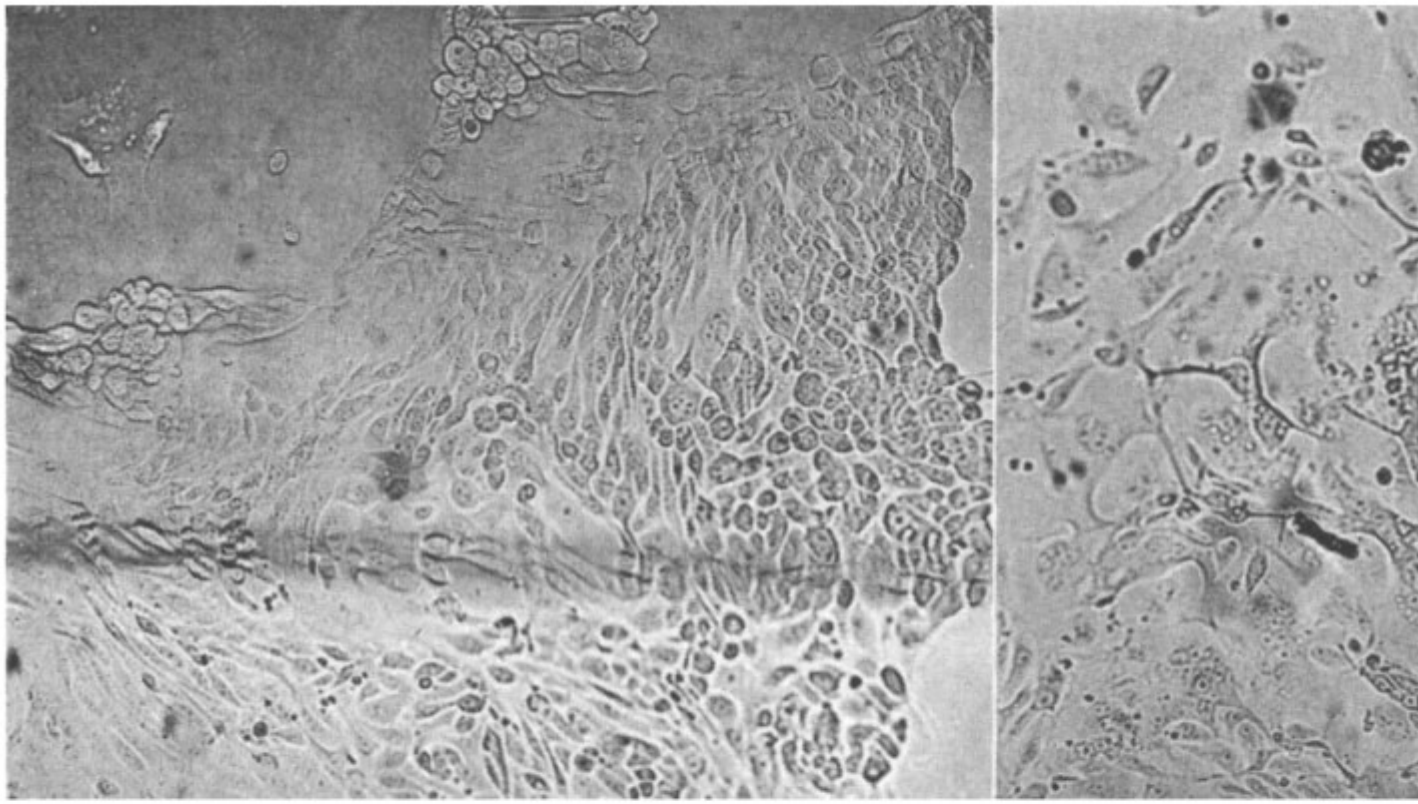

Fig. 1.

FIG. 1. Outgrowth of normal human kidney cells in an uninoculated r
Control for cultures shown in Fig. 2 and 3.
FIG. 2. Area of syncytial giant cells with small cytoplasmic vacuoles
faint nuclear outlines. 9th day after inoculation; 7th passage, a
(×130).

In Summary:

- The original measles results of different investigators were often at variance or directly contradictory
- Enders claims that monkeys, especially rhesus macaques, are moderately susceptible to experimental inoculation
- Shaffer and their collaborators have provided evidence **suggesting** that the agent which passed through bacteria-retaining filters could be maintained indefinitely **in serial passages** in the developing chick embryo
- However, egg passage in the hands of Shaffer and his coworkers **regularly appeared to alter the pathogenicity of the agent** for man as indicated by the

development of a mild and much modified disease following the inoculation of egg adapted materials into susceptible children

- The above work by Shaffer would seemingly indicate that the type of culture (tissue, cell, embryo) has a profound impact on the results gained from the experiments
- Since 1943, no progress was made with measles research which Enders attributed to the lack of a convenient laboratory method for the demonstration of the presence of the agent which induced no recognizable changes in eggs or cultures of chick tissues
- Repeated attempts by Shaffer to demonstrate a serologic reaction, such as complement fixation, using materials from the infected chick embryo failed
- Thus, Enders steps in with his work to claim that agents had been demonstrated that can be maintained in serial passage in tissue cultures and which induce distinctive cytopathic changes in renal epithelial cells
- Patients were asked to gargle with 10-15 ml of sterile neutralized fat-free milk
- Certain specimens from the throats of younger children were obtained by cotton swab previously moistened in milk
- After swabbing the throat, the swab was immersed in 2 ml of milk
- Penicillin, 100 u/ml, and streptomycin, 50 mg/ml. were added to all throat specimens which were then centrifuged at 5450 rpm for about one hour
- Supernatant fluid and sediment resuspended in a small volume of milk were used as separate inocula in different experiments in amounts varying from 0.5 ml to 3.0 ml
- About 10 ml of blood immediately after withdrawal were placed in tubes containing 2 ml of 0.05% solution of heparin
- After addition of antibiotics as described above, 10% fecal suspensions were prepared by grinding the material in bovine amniotic fluid medium
- Trypsinized human and rhesus monkey kidney were later employed for “isolation” of agents and their passage

- The culture medium consisted of bovine amniotic fluid (go %), beef embryo extract (50/0), horse serum (5%), antibiotics, and phenol red as an indicator of cell metabolism
- Soybean trypsin inhibitor was added to this medium unless it was used for the cultivation of human and monkey kidney
- **Serial passage** of the various strains was accomplished as routine by removal of the culture medium between the 4th and the 16th day after inoculation and immediate transfer of 0.1 ml to each of a number of fresh cultures

Related posts on the effects of Passaging on culture:

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- Enders observed cytopathogenic effects (CPE) after 7 days of culture and determined that these changes presented a characteristic appearance not associated definitely with a "virus" thus he assumed it was evidence of measles being present in the cell culture
- Under electron microscope examination, since in these and other preparations that have been examined subsequently no clear unstained zone has been observed between the chromatin and this acidophilic mass, **it cannot be asserted that the latter represents an intranuclear inclusion body of the type characteristically associated with "viral" infections**
- However, Enders states it can be used as criterion for determining infection
- He then states it should be emphasized that the changes as just depicted are encountered in cultures that have been **incubated for relatively prolonged periods** (e.g. 14-21 days)
- When the interval between inoculation of the agent and examination of the stained cells (e.g. 4 days) **is shorter**, margination of the chromatin may be incomplete or inapparent

- Once again, this shows that the culture conditions and the length of time greatly influence the interpretation of the results obtained
- Monkey kidney was the only other tissue employed that has yielded a growth of cells in which the characteristic changes described above have been definitely observed following inoculation of “virus”
- However, regarding the CPE observed, Enders admits that cytopathic effects which superficially resemble those resulting from infection by the measles agents **may possibly be induced by other “viral” agents present in the monkey kidney tissue or by unknown factors**, thus showing that not only is the CPE not specific but can be caused by factors other than a “virus”

Related posts on other factors which can cause CPE:

The Cytopathic Effect

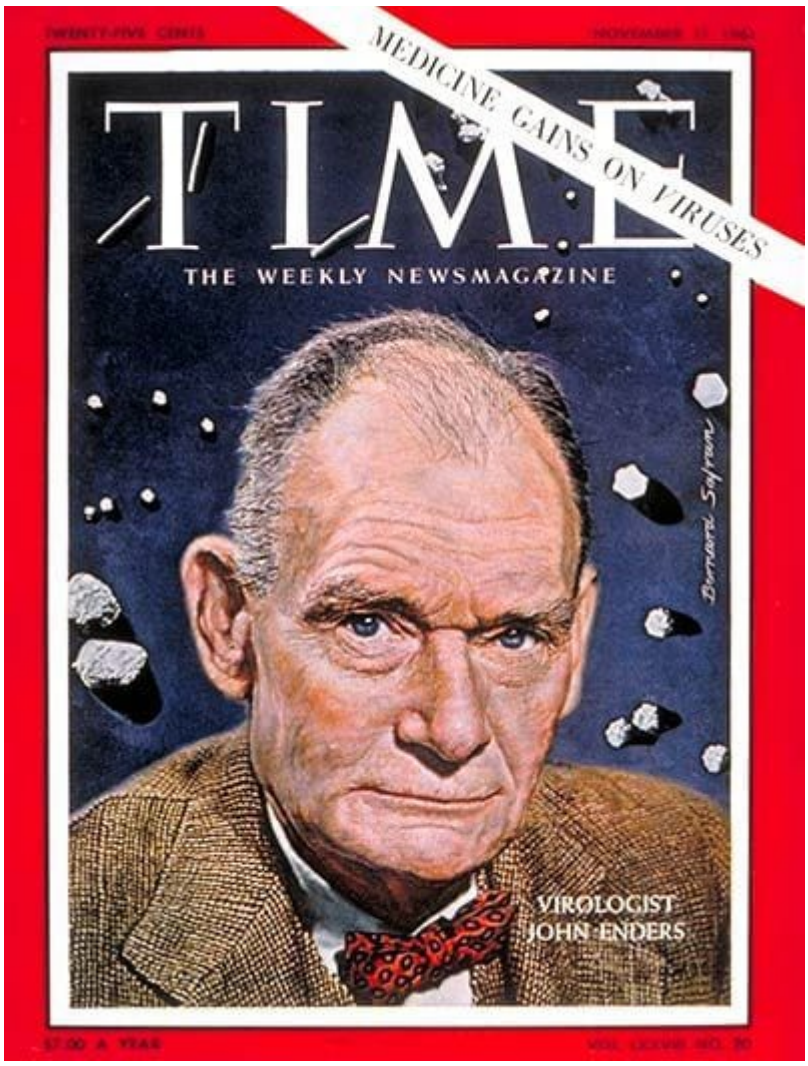
Creating the Cytopathic Effect

- Two other agents were also “isolated” while the present work was in progress which, to Enders, appeared unrelated to those he described
- The cytopathic changes it induced in the unstained preparations **could not be distinguished** with confidence from the “viruses” isolated from measles
- Enders states that obviously the possibility of encountering such agents in studies with measles should be constantly kept in mind
- This further demonstrates that the material Enders worked with was not properly purified nor was the measles “virus” ever separated from everything else as there were other “agents” present
- Enders claims that great caution should be exercised in the interpretation of any new claims that the “virus” has been propagated in other hosts or systems outside of monkeys
- He also states that the results that he has summarized must be subjected to the most critical analysis

- Enders believed that the indirect evidence he presented supported the hypothesis that the “viruses” he described are responsible for the disease
- Enders proceeds to shoot his own cytopathogenic evidence in the foot when he states that while there is no ground for concluding that the factors in vivo (within a living organism) are the same as those which underlie the formation of giant cells and the nuclear disturbances in vitro (outside the living organism), the appearance of these phenomena in cultured cells is consistent with the properties that a priori **might be associated with the “virus” of measles**
- Even though he relied on antibody evidence to make the case he “isolated” a measles “virus,” Enders states more tests are desirable **in order to eliminate any remaining doubt concerning the specificity of these serologic reactions**
- Enders admits they obtained considerable **indirect** evidence
- He also admits 2 remaining criteria are needed to be carried out and met:

1. The production of measles in the monkey and in man with tissue culture materials after a number of passages in vitro sufficient to eliminate any “virus” introduced in the original inoculum
2. The recovery of the “virus” from the experimental disease in these hosts should then be accomplished

- Enders concludes that his indirect evidence support the **presumption** that this group of agents is composed of representatives of the “viral” species responsible for measles



Medicine “gains” on “viruses.” Really?!?

It is clear to see upon reading this that there can be no claims of purified/isolated measles “virus” as multiple alterations and additions to the original samples occurred. For starters, the throat swabs were immediately placed in milk or were taken by cotton swabs doused with milk. Antibiotics were then added to these milk swab samples. Blood samples were placed in tubes containing heparin which is toxic to cells. Fecal samples were ground up and added to bovine amniotic fluid serum. Numerous chemicals/compounds were used during the “isolation” process. Trypsinized human or monkey kidneys cells were used and trypsin has been shown to have negative effects on the kidneys and can be full of contaminants. Bovine amniotic fluid, beef embryo extract, horse serum, antibiotics, phenol red, and in some cases soybean trypsin inhibitors, were added to the culture. The addition of various cell-altering chemicals, compounds, animal DNA, etc is the exact opposite of purification/isolation.

The cytopathic changes Enders observed which led him to conclude a “virus” was present occured after culturing for 7 days. Enders stated that the cytopathic changes (CPE) seen in monkey kidney cells resembles that of human kidney cells and that they can be used in place of the human cells going forward for culturing just as they are regularly used today. However, just observing CPE in cell culture does not mean a “virus” is present nor that it is to be blamed for the effect. Any of the toxic compounds alone which were added to the culture could have caused these changes. There is no reason to assume a “virus” was the cause of cell death. Enders even admits that the CPE he observed may not be due to a measles “virus” but could be the result of different “viruses” or other as of yet unknown factors. Thus, his pivotal evidence was in fact no evidence at all.

Enders also admitted to other agents being “isolated” along with measles and that they could not distinguish any difference between the CPE they claim was caused by a measles “virus” with the CPE produced by one of the other agents. This is just further evidence that these were not purified/isolated samples and that they assume that whatever “virus” they believe is present must adhere to certain CPE changes even though there are other agents which can produce the same effect. The CPE observed is not specific to any “virus.”

Enders also made some startling revelations in his paper which tend to throw his own evidence down the drain. First, he claims that there is no grounds for stating that what happens **IN VITRO** (in a lab) has any relation to what happens **IN VIVO** (within a living organism). However, everything he did and the resulting evidence he claimed originated from experiments done outside of a living organism.

Second, Enders admits that they only collected **INDIRECT** evidence supporting a role in the agents he studied yet further experiments were needed in order to prove he actually acquired a measles “virus.” This included actually seeing if the agent can produce measles in a human or monkey and whether the measles “virus” can be recovered from either of them. Thus this paper is not proof of a measles

“virus” at all. If that wasn’t enough to make this point absolutely clear, Enders conclusion definitely will:

“The findings just summarized **support the presumption** that this group of agents is composed of representatives of the viral species responsible for measles.”

This is the seminal measles work and it does not offer any proof of the existence of a measles “virus.” It presumes (i.e. **to suppose to be true without proof**) a “virus.” Everything built upon this fraudulent paper is therefore fraudulent as well, which is the very nature of “virology” and “science” today.