

PSEUDOSCIENTIFIC VIROLOGY

A BRIEF SUMMARY

PRESENTATION *by*

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SECTIONS

TITLE	PAGE NO.
1. FAILED TRANSMISSION STUDIES FOR FEW DIS-EASES.	3-18
2. FOI RESPONSES: NO EVIDENCE FOUND FOR ANY ALLEGED "PATHOGENIC VIRUS".	19-29
3. CONTROLS AND NON-SPECIFIC CYTOPATHIC EFFECT (CPE).	30-45
4. MISINTERPRETATION OF ALLEGED "PATHOGENIC VIRUSES".	46-79
5. REFERENCES.	80-89

1. FAILED TRANSMISSION STUDIES FOR FEW DIS-EASES.

Results.—In only one instance (Experiment 2 (a)) was any reaction observed in which a diagnosis of influenza could not be excluded, and here a mildly inflamed throat seemed the more probable cause of the fever and other symptoms. Nothing like influenza developed in the other volunteers.

In no instance was a clinical case of influenza produced.

Three of the volunteers who received unfiltered nasopharyngeal secretions became ill with acute lacunar tonsillitis.

Attempts to transmit influenza by means of cultures of Pfeiffer's bacillus and of Mather's streptococcus were unsuccessful.

Pfeiffer's bacillus is found in the throats of many people who are free from influenza, but shows a tendency to multiply and become predominant during an attack of the disease.

We also employed two species of new world monkeys, one belonging to the genus *Cebus* and including *Capuchinus*. The larger ring tail proved susceptible and the smaller did not, although five individuals of the latter were inoculated. The question whether the catarrhine are more uniformly susceptible than the platyrrhine species is an interesting one.

In this connection brief reference should be made to other species of animals employed for inoculation. Besides many guinea-pigs and rabbits, one horse, two calves, three goats, three pigs, three sheep, six rats, six mice, six dogs, and four cats have had active virus introduced in the brain but without causing any appreciable effect whatever. These animals have been under observation for many weeks.

strable in the secretions from the nose and throat. Rosenau, Sheppard and Amoss therefore injected 18 monkeys with the nasal and buccal secretions obtained from 18 persons who were suffering with the disease at the time, or in the stage of convalescence, or from persons suspected of acting as carriers. These results were negative. At the same time Straus of New York had a series of negative results, and other American workers were also unable to find the virus where we assumed it should be. These negative results seemed to us to have positive significance, and was the first definite indication that we were upon the wrong trail.

The fluids from forty patients with poliomyelitis were centrifuged at high speed for three quarters of an hour. As our clinical work consists chiefly of the diagnosis of suspicious cases, these fluids were derived from cases early in the disease, most of them in the preparalytic stage. The sediments, amounting to 1 c.c. of very turbid fluid, were injected intracerebrally into Rhesus 23. Culture tubes of glucose ascitic agar inoculated with some of the sediment remained sterile.

No effects were noted, and after an observation period of two months, this animal was inoculated with

One experiment was made to isolate the virus from the feces of a patient with an acute case. The feces of

Patient 9 were mixed with salt solution on the same day that the nasal swab was taken. The mixture was filtered through a Berkefeld filter and the filtrate centrifuged. Four c.c. of the sedimented portion were injected intracerebrally into a monkey. The animal died of marasmus after four weeks. The autopsy revealed no cause of death. An emulsion of his brain and cord was inoculated into a second monkey with negative result. This experiment, so far as we are aware, is the only one in which an attempt has been made to demonstrate the virus in the human feces.

Summary and conclusion.—As there were no cases after the inoculation in any group, the value of the prophylactic measure cannot be gauged. It is evident that neither the vesicular fluid nor the serum of the patients collected on the fourth day, diluted and filtered as above, was capable of producing the disease after an intradermal inoculation. Amongst the vesicular-fluid group about 8 per cent had headaches in addition to the erythema, the other groups being free from this symptom.

The materials employed for inoculation were derived from patients in the early stages of scarlet fever. This was rendered

The third class of scarlatinous material was composed of (a) urine obtained under sterile precautions and found sterile by culture; (b) whole blood collected from a vein in paraffin-coated iced tubes, and injected, before clotting, intravenously into a monkey; (c) defibrinated blood; (d) sputum filtrates (Berkefeld); (e) filtrates of broth cultures of streptococcus from scarlatinous throats; (f) blood from scarlet fever cases mixed with ascitic broth and incubated at 37° C.

The routes of introduction were through the mucous membranes, skin, stomach, joint cavities, blood stream, peritoneum, and brain. It seemed possible that repeated inoculations at three week intervals over a long period might bring about a state of hypersensitiveness and so lead to infection. Consequently several monkeys were treated in that manner. The animals stood the inoculation well and showed no reaction of any kind. In this series most of the inocula-

SUMMARY

The attempt to produce scarlet fever in monkeys and baboons by the faucial inoculation of streptococcus cultures or unfiltered exudates and discharges from scarlet-fever cases, as well as the intraperitoneal injections of filtrates of such exudates and discharges and of large quantities of blood, gave negative results.

CONCLUSION

This brief review of the recorded attempts to produce scarlet fever experimentally in man reveals that it is exceedingly doubtful whether a single positive result has been obtained. In view of the ease with which scarlet fever appears to be transmitted under natural conditions and the not infrequent occurrence of surgical scarlet fever, the failure of the efforts at experimental transmission is a perplexing puzzle that awaits solution.

We attempted to produce experimental scarlet fever in guinea pigs, mice, rabbits, dogs, pigeons, and small white pigs.

repeat their work. The animals were inoculated with blood from early cases, and with pure cultures of many different bacteria obtained from scarlet fever, also with ground-up organs from post mortems, and with mucus freshly obtained from the throat early in the disease.

In this long series of animal inoculations, an occasional rash was obtained, and, less frequently, desquamation. But no one organism produced these symp-

toms constantly in any species; so the most essential evidence for the determination of the etiology of scarlet fever was still lacking. We decided that animals

Healthy young adults who said that they had not had scarlet fever were chosen. In the first series of human inoculations, reported in 1921, volunteers were inoculated with fresh whole blood

and fresh blood serum from acute cases of scarlet fever; also with filtered throat mucus from early cases. The results of these inoculations were negative. Then

We found that the scarlet fever streptococci produce a toxin. When this toxin is absorbed into the blood, it produces the rash. The toxin is obtained by inoculat-

It is remarkable that Sellards was unable to produce this highly infectious disease by means of the blood or the nasal secretion of infected individuals. Not long ago, however, I had a similar experience with varicella (*Am. J. Dis. Child.* **16**:34 [July] 1918). Thus we are confronted with two diseases—the two most infectious of the endemic diseases in this part of the world—which we are unable to transmit artificially from man to man. The result was most surprising in regard to chickenpox, and if the same rule holds good for measles it would seem as if a basic principle must be involved. Evidently in our experiments we do not, as we believe, pursue nature's mode of transmission; either we fail to carry over the virus, or the path of infection is quite different from what it is commonly thought to be.

CONCLUSIONS

1. The question of the transfer of measles from man to man by the injection of a patient's blood is entirely reopened by the eight successive negative inoculations recorded in this paper.

to. I made the experiments upon seventeen people between the ages of fifteen and thirty years, but in no instance could a case of consumption, scarlet fever, small-pox or diphtheria be produced.

These experiments were made in the following manner: I sprayed the poisons of diphtheria, small-pox, scarlet fever, or consumption into the throat, nose, or had them breathe them into the lungs, repeating the experiment in most cases every one or two weeks for months, with the result that no disease could be developed.

usual soreness as in vaccination. Then I took into my system the typhoid bacilli, and no typhoid fever making its appearance, I repeated the experiment with diphtheria germs, without the least perceptible effect.

“In order to make the experiments still more complete, I cultivated the germs of diphtheria and glanders until there could be no doubt of their virility and took them into my system in the presence of two reputable physicians. The outcome was precisely the same as before.

“Then I made the greatest trial of all. In the presence of twenty-five physicians I took, first, the bacilli of typhoid into the stomach enclosed in gelatine capsules; and, second, the bacilli of diphtheria by both the vaccination method and subcutaneous inoculation.

“Examinations were afterwards made by the physicians referred to of the pulse, the temperature and of the respiration, and it was unanimously declared that these inoculations produced no greater effect upon me than might have been expected from a like quantity of water.

abdominalis—the germ of typhoid fever; the **bacillus diphtheriae**—the germ of diphtheria, and the **bacillus tuberculosus**—the germ of pulmonary consumption, and tubercular diseases in general. **These germs were introduced in various ways**; first, by application to denuded surfaces, as in vaccination; second, by ingestion, or swallowing germ-laden “cultures”; third, by injecting germ-laden “cultures” into the bowels; fourth, by subcutaneous injection, or hypodermic inoculation; fifth, by insufflation—drawing into the lungs a powder made from carefully dessicated sputum of a patient who was dying of consumption, and which was heavily laden with tubercle bacilli, there being an average of twenty-five germs visible within the field of the microscope.

Experiments of this kind were made on six different occasions, and with no other precaution than that of seeing that the body was practically free of such evidences of the presence of pathogen as the existence of congestion and catarrhal transudation. In every experiment, except the first, the germs were furnished and their introduction was supervised by reputable physicians. On the **last three occasions** the experiments were made in the presence of about **twenty-five physicians**, who declared upon due investigation that not the slightest symptom of morbid action of any kind could be detected—**that there was no change of temperature, no enlargement of the glands and no inflammatory action.**

**2. FOI RESPONSES: NO
EVIDENCE FOUND FOR ANY
ALLEGED “PATHOGENIC
VIRUS”.**

2.1.

This is a formal request for access to general records, made under the *Freedom of Information Act*.

Description of Requested Records:

All studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) that describe anyone on Earth finding and **purifying** any "**avian influenza virus**" directly from a sample (i.e. bodily fluid/tissue/excrement) taken from any diseased host (animal or human) where the sample taken from the host was not first combined with any other source of **genetic** material (i.e. a cell line; fetal bovine serum).

Please note that I do not require and do not want studies/reports where researchers failed to **purify** an alleged "virus" from the sample and instead:

- cultured something, and/or
- performed an amplification test (i.e. PCR), and/or
- fabricated a "genome", and/or
- produced electron microscopy images of unpurified things.

For further clarity, please note I am already aware that according to virus theory a "virus" requires host cells in order to replicate, and that a strict application of Koch's Postulates would not be possible even if theoretical "viruses" existed. I do not require records describing the **replication** of an alleged "virus" without host cells, or strict fulfillment of Koch's Postulates, or a suspected "virus" floating in a vacuum, or private patient records.

I simply require records that describe **purification** (**separation** from everything else in the sample that was taken from the host, as per standard laboratory practices for the purification of other very small things).

A search of our records failed to reveal any documents pertaining to your request..

This is a formal request for access to general records, made under the *Freedom of Information Act*.

Description of Requested Records:

Please note: *this request is very similar to another request that I submitted on March 1, 2021 (21-00794-FOIA) where I had **specified** purification via maceration, filtration and use of an ultracentrifuge and received a "no records" response. The difference with this new request is that it does **not** specify maceration, filtration and use of an ultracentrifuge; it mentions filtration, ultracentrifugation and chromatography only by way of an example.*

All studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) describing the **purification** (i.e. via filtration, ultracentrifugation and chromatography) of any **"Ebola virus" said to have caused disease in humans (i.e. the "Ebola virus", "Sudan virus", "Tai Forest virus", "Bundibugyo virus")**, where the sample was not first combined with any other source of **genetic** material (i.e. a cell line; fetal bovine serum).

Please note that I am not requesting studies/reports where researchers failed to **purify** the suspected "virus" and instead:

- cultured something, and/or
- performed an amplification test (i.e. PCR), and/or
- fabricated a "genome", and/or
- produced electron microscopy images of unpurified things.

For further clarity, please note I am already aware that according to virus theory a "virus" requires host cells in order to replicate. I am **not** requesting records describing the **replication** of a "virus" without host cells, nor records that describe a suspected "virus" floating in a vacuum or a strict fulfillment of Koch's Postulates, or private patient records. I simply request records that describe "its" **purification (separation)** from everything else in the patient sample, as per standard laboratory practices for the purification of other very small things).

Please note that my request includes any study/report matching the above description, **authored by anyone, anywhere.**

A search of our records failed to reveal any documents pertaining to your request. Specifically, the National Center for Emerging and Zoonotic Infectious Diseases states for the specified virus, the "procedure asked about in this FOIA request is not a methodology or procedure that the branch utilizes or has ever implemented. We routinely, per protocol, place samples on cell culture post sample receipt."

Description of Requested Records:

1. All studies/reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) that scientifically prove the existence of the alleged **herpes virus (herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2))**;

Note:

Scientific proof is NOT

- Opinions
- Speculation
- Review papers
- Descriptive papers

Scientific proof requires

- Use of the scientific method
- Repeatable and falsifiable hypotheses that have been tested using valid, controlled experiments where only 1 variable differs between the experimental and control groups
- In this case, the 1 manipulated variable would be the presence/absence of purified particles suspected of being a "virus"
- Consistent results from valid, controlled experiments (i.e. identical "genomes", consistent in vivo effects)

2. **If the CDC has no studies responsive to #1 above**, then please indicate such explicitly, and provide all studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) describing the **purification** of particles that are alleged to be this virus, directly **from bodily fluid/tissue/excrement or from a cell culture, with purification confirmed via EM imaging** (the images must be available as well).

Please note that I am not requesting studies/reports where researchers failed to **purify** the suspected "virus" and instead:

- cultured an unpurified sample or other unpurified substance, and/or
- performed an amplification test (i.e. a PCR test), and/or
- created an in silico "genome", and/or
- produced electron microscopy images of unpurified things.

A search of our records failed to reveal any documents pertaining to your request. Program does suggest you may wish to contact the National Institutes of Health (NIH) at the following address:

“HERPES VIRUS”

This is a formal request for access to general records, made under the *Freedom of Information Act*.

Description of Requested Records:

*[Please note: this request is very similar to another request that I submitted on March 1, 2021 (#21-00793-FOIA) where I had **specified** purification via maceration, filtration and use of an ultracentrifuge and received a "no records" response. The difference with this new request is that it does **not** specify maceration, filtration and use of an ultracentrifuge; instead it mentions filtration, ultracentrifugation and chromatography by way of an example.]*

All studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) describing the **purification** of any "**HIV**" aka "**human immunodeficiency virus**", directly from a sample taken from an diseased human (**with AIDS symptoms**) where the patient sample was not first combined with any other source of **genetic** material (i.e. monkey kidney cells aka Vero cells or any other cell line; fetal bovine serum).

Please note that I am not requesting studies/reports where researchers failed to **purify** the suspected "virus" and instead:

- cultured something, and/or
- performed an amplification test (i.e. PCR), and/or
- fabricated a "genome", and/or
- produced electron microscopy images of unpurified things.

I am already aware that according to virus theory a "virus" requires host cells in order to replicate. I am not requesting records describing the **replication** of a "virus" without host cells, or records that describe a suspected "virus" floating in a vacuum, or private patient records.

I simply request records that describe **purification** of the alleged virus (**separation** from everything else in the patient sample as per standard laboratory practices for the purification of other very small things).

A search of our records failed to reveal any documents pertaining to your request.

Description of Requested Records:

*[Please note: this request is very similar to another request that I submitted on April 28, 2021 (#21-01193-FOIA) where I had **specified** purification via maceration, filtration and use of an ultracentrifuge and received a "no records" response. The difference with this new request is that it does **not** specify maceration, filtration and use of an ultracentrifuge; it only mentions filtration, ultracentrifugation and chromatography by way of an example.]*

All studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) describing the **purification** (i.e. via filtration, ultracentrifugation and chromatography) of any "**HPV**" aka "**Human Papillomavirus**" said to have caused disease in humans, directly from a sample taken from a diseased human where the patient sample was not first combined with any other source of **genetic** material (i.e. monkey kidney cells aka Vero cells; fetal bovine serum).

Please note that I am not requesting studies/reports where researchers failed to **purify** the suspected "virus" and instead:

- cultured something, and/or
- performed an amplification test (i.e. PCR), and/or
- fabricated a "genome", and/or
- produced electron microscopy images of unpurified things.

For further clarity, please note I am already aware that according to virus theory a "virus" requires host cells in order to replicate, and I am **not** requesting records describing the **replication** of a "virus" without host cells.

Further, I am **not** requesting records that describe a suspected "virus" floating in a vacuum or private patient records; I am simply requesting records that describe its **purification (separation)** from everything else in the patient sample as per standard laboratory practices for the purification of other very small things).

A search of our records failed to reveal any documents pertaining to your request.

I require access to general records, as per the *Freedom of Information Act*.

Description of Requested Records:

All records in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) that 1) reveal the origin of image #22664 posted on the CDC's website (<https://phil.cdc.gov/Details.aspx?pid=22664>; "Content Providers(s): CDC/ Cynthia S. Goldsmith") that allegedly shows "a monkeypox virion", and/or 2) describe the methodologies used to obtain said image.

If any records match the above description of requested records and are currently available to the public elsewhere, please provide enough information about each record so that I may identify and access each one with certainty (i.e. title, author(s), date, journal, where the public may access it). Please provide URLs where possible.

A search of our records failed to reveal any documents pertaining to your request. Records subject to the Freedom of Information Act are maintain in a system of records within the agency control. However, the program has provided information on the photo that is the subject of your request. The program has reported the following:

I require access to general records, as per the *Freedom of Information Act*.

Description of Requested Records:

All studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) that scientifically prove the existence of any alleged "**polio virus**".

Note:

Scientific proof is NOT

- Opinions
- Speculation
- Review papers
- Descriptive papers

Scientific proof requires

- Repeatable and falsifiable hypotheses that have been tested using valid, controlled experiments where only 1 variable differs between the experimental and control groups;
- In this case, the 1 manipulated variable should be the presence/absence of the particle alleged to be "the virus"
- Consistent results from the valid controlled experiments (i.e. identical "genomes")

Records that do not describe the testing of falsifiable, repeatable hypotheses regarding the existence of this alleged "virus" (meaning the existence of the alleged particle and its alleged causation of disease) are disqualified from my request.

A search of our records failed to reveal any documents pertaining to your request. Additionally, program provides as follows.

Polio was shown to be caused by a virus over 100 years ago, by transfer of filtered material from a fatal polio case to non-human primates (Landsteiner and Popper, 1908). Polio was the first human disease shown to be caused by a virus ("filtrable agent" was the term at the time). Virus isolation in cell culture was first published in 1949 (also Polio).

"POLIO VIRUS" [Also read, [Ref. 5.3.](#)]

This is a formal request for access to general records, made under the *Freedom of Information Act*.

Description of Requested Records:

All studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) describing the **purification** (i.e. via filtration, ultracentrifugation and chromatography) of any "**rabies virus**", directly from a sample taken from a diseased host (i.e. bat, raccoon, skunk, fox, dog, human, etc.), where the sample was not first combined with any other source of **genetic** material (i.e. a cell line; fetal bovine serum).

Please note that I am **not** requesting studies/reports where researchers failed to **purify** the suspected "virus" and instead:

- cultured something, and/or
- performed an amplification test (i.e. PCR), and/or
- fabricated a "genome", and/or
- produced electron microscopy images of unpurified things.

I am already aware that according to virus theory a "virus" requires host cells in order to replicate. I am **not** requesting records describing the **replication** of a "virus" without host cells, or records that describe a suspected "virus" floating in a vacuum, or a strict fulfillment of Koch's Postulates, or private patient records.

I simply request records that describe "its" **purification** (**separation** from everything else in the patient sample, as per standard laboratory practices for the purification of other very small things).

A search of our records failed to reveal any documents pertaining to your request. Specifically, the Poxvirus and Rabies branch (PRB) within NCEZID's Division of High Consequence Pathogens and Pathology, states as follows:

This is a formal request made under Canada's *Access to Information Act*.

Description of Requested Records:

All records in the possession, custody or control of the Public Health Agency of Canada (PHAC) describing the isolation of a SARS-COV-2 virus, directly from a sample taken from a diseased patient, where the patient sample was not first combined with any other source of genetic material (i.e. monkey kidney cells aka vero cells; liver cancer cells).

Please note that I am using "isolation" in the every-day sense of the word: *the act of separating a thing(s) from everything else*. I am not requesting records where "isolation of SARS-COV-2" refers instead to:

- the culturing of something, or
- the performance of an amplification test (i.e. a PCR test), or
- the sequencing of something.

Please also note that my request is not limited to records that were authored by the PHAC or that pertain to work done by PHAC. My request includes any sort of record, for example (but not limited to) any published peer-reviewed study that PHAC has downloaded or printed.

As requested, The Public Health Agency of Canada has further discussed with the program area and requested clarification of the records that were provided in response to the request above.

Your request has resulted in a “No Records Exist”, because of the way that you have formulated your request. The isolation of the virus is not completed without the use of another medium, therefore we have no records that would show this process taking place. It is important to understand the following: The gold standard assay used to determine the presence of intact virus in patient samples is viral isolation in cell culture. With this

I require access to general records, as per your duty under the *Freedom of Information Act*.

Description of Requested Records:

All studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) describing the **purification** of the "**Zika virus**" said to have caused disease in humans, directly from a sample taken from a diseased human, **via any method** where the patient sample was not first combined with any other source of **genetic** material (i.e. monkey kidney cells aka Vero cells; fetal bovine serum).

Please note that I am not requesting studies/reports where researchers failed to **purify** the suspected "virus" and instead:

- cultured an unpurified sample or other unpurified substance, and/or
- performed an amplification test (i.e. a PCR test) on all the RNA from a patient sample or from a cell culture, or on genetic material from any unpurified substance, and/or
- sequenced the total RNA from a patient sample or from a cell culture or from any unpurified substance, and/or
- produced electron microscopy images of unpurified things.

For further clarity, please note I am already aware that according to virus theory a "virus" requires host cells in order to replicate, and I am **not** requesting records describing the **replication** of a "virus" without host cells.

Further, I am **not** requesting records that describe a suspected "virus" floating in a vacuum; I am simply requesting records that describe its **purification** (**separation** from everything else in the patient sample, as per standard laboratory practices for the purification of other small things).

A search of our records failed to reveal any documents pertaining to your request.

3. CONTROLS AND NON-SPECIFIC CYTOPATHIC EFFECT (CPE).

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

*A cytopathogenic agent recovered
from an apparently normal
kidney tissue culture*

A cytopathogenic agent was originally encountered in a control uninoculated culture tube of rhesus kidney tissue 7 days after the beginning of incubation. The first sign noted was a small circular hole devoid of cells in a continuous sheet of cells. Surrounding this clear area was

sue-culture safety testing of the vaccine as the occurrence of these viruses has in many instances invalidated tests for poliovirus, making retesting necessary and thereby delaying the release of vaccine. There has also been the problem of proving in some cases that the contaminating "simian virus" isolated during a vaccine safety test was not in the vaccine sample under test. In general, however, this has not been a problem since many of these agents have been recovered from normal or uninoculated control cultures. The likelihood of the contaminating S.V. agent being in the vaccine sample has also in many instances been diminished by a statistical

In recent years, with the increased use of tissue cultures prepared from monkey kidney cells, a new group of viruses has come into recognition. The presence of these agents is made known by the cytopathic effect which they produce in uninoculated or control cultures. The combined observations of workers in several laboratories indicate that these agents, as yet unclassified, are unaccountably present in the kidney tissues (or blood elements) of the apparently normal, healthy monkeys from which the cultures are derived (1-3).

The existence of monkey-foamy-agent in kidney tissue obtained from so-called normal monkeys has been described by several investigators¹. The hazard of encountering foamy-agent in experiments carried out with measles virus in such cultures has been indicated by *Enders* and *Peebles*². Cytologic criteria cannot be applied for their distinction in the living culture since both agents produce similar effects. On staining however, characteristic intranuclear inclusions are absent in changes induced by the foamy-agent. Early in the study of measles virus in monkey kidney tissue, a second agent was encountered in uninoculated monkey kidney cultures, which was, in its cytopathic capacity, indistinguishable from measles virus and provisionally referred to as monkey-intra-nuclear-inclusion-agent (MINIA³). Because of the possibility of encountering foamy-

208

F. L. BLACK, M. REISSIG, AND J. L. MELNICK

or cynomolgus monkey kidney is the easier for most laboratories to obtain. Unfortunately, early in the course of this work it was found that agents which induced cytopathic effects superficially resembling that of measles virus occurred in uninoculated cultures. The original observations have since been confirmed and extended by Rustigian *et al.* (1955), Ruckle (1958), and Brown (1957). The changes induced by one of the commonest of these agents develop from one to three weeks after preparation of the cultures; they are characterized by the formation of multinucleated giant cells, although intranuclear inclusions cannot be demonstrated. The

A SECOND IMMUNOLOGIC TYPE OF SIMIAN FOAMY VIRUS: MONKEY THROAT INFECTIONS AND UNMASKING BY BOTH TYPES

PAUL B. JOHNSTON*

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During the last 6 years there have been many reports of isolation of simian "foamy" virus from "uninoculated" kidney cell cultures of rhesus and cynomolgus monkeys (Enders and Peebles 1954; Rustigian et al, 1955; Henle and Deinhardt, 1955; Hotta and Evans, 1956; Brown, 1957; Falke, 1958; Ruckle, 1958a; Endo et al, 1959) and from African monkeys and baboons (Hsiung et al, 1958; Lepine and Paccaud, 1957).

used (Melnick, 1956), and the cell inoculum was standardized by centrifuging at 500 rpm for 10 minutes to measure the packed cell volume. Using the ratio of 1 ml of packed cells to 100 ml of Eagle's medium with 10% heat-inactivated calf serum and 0.5 ml per culture, all attempts with *Macaca cyclopsis* gave cell sheets without granulation within 5 days. The medium was changed twice a week using 1-ml volumes and the sodium bicarbonate concentration was increased as the cells grew older. At the time of foamy virus harvesting the sodium bicarbonate concentration was 0.24%.

Presence of adventitious agents. During prolonged incubation (2 weeks or more) of uninoculated chimpanzee kidney tissue cultures, the cells frequently exhibited changes similar to changes caused by the growth of viruses; the maintenance medium from several of these cultures was stored at -70°F for further study. No hemagglutinins were detected in uninoculated cultures.

iii. RNA and RNA directed DNA polymerase in human leukemic cells. 70S poly(A)-containing RNA is found in lymphocytes in a particulate cytoplasmic fraction having a density of 1.16. An endogenous and completely RNA-dependent (i.e., RNAase-sensitive) DNA polymerase activity was also found in this fraction. The activity was obtained from leukemic blood lymphocytes (and myeloblasts) and from PHA stimulated (but not in unstimulated) normal human blood lymphocytes. Analysis

authors of the *Science* paper, were aware that RT is not specific to retroviruses and can be found even in normal cells. In the early 1970s Gallo and his associates proved that cultures of leukaemic cells transcribe the An·dT₁₅ template-primer as does material banding at 1.16 g/ml originating from "PHA stimulated (but not unstimulated) normal human blood lymphocytes" [30]. In 1975 an

[Also refer, [Ref. 5.2.](#)]

SUMMARY The aetiology of the inflammatory bowel diseases, Crohn's disease, and ulcerative colitis, is still obscure. A viral aetiology has been proposed, based in part on reports that filtrates prepared from tissues of patients with inflammatory bowel disease induce cytopathic effects in tissue culture cells. Our attempts to culture viruses in many cell lines from filtrates prepared from the tissue of 95 patients have been negative, except for one case in which cytomegalovirus was isolated from the tissue of a Crohn's disease patient. Our studies confirm previous reports that intestinal tissue filtrates induce cytopathic effects in inoculated cell cultures, but the effect we observed is non-specific; cytopathic effects were induced in most cell lines tested and with similar frequency irrespective of whether the intestinal filtrates were prepared from Crohn's disease patients, ulcerative colitis patients, or non-inflammatory bowel disease controls. Electron microscopy studies of tissue culture cells exhibiting cytopathic effects have not revealed virus particles. Characterisation of the cytopathic effect inducing factor showed that it was incapable of serial passage in tissue culture, too small to be a conventional virus, resistant to inactivation by ultraviolet light, and heat stable. Our results suggest that the observed cytopathic effect was caused by a non-replicating cytotoxic factor, or factors, released from intestinal tissues of both inflammatory bowel disease and non-inflammatory bowel disease patients.

EVIDENCE FOR PRENATAL TRANSFER OF RABIES VIRUS IN THE MEXICAN FREE-TAILED BAT (*TADARIDA BRASILIENSIS MEXICANA*)

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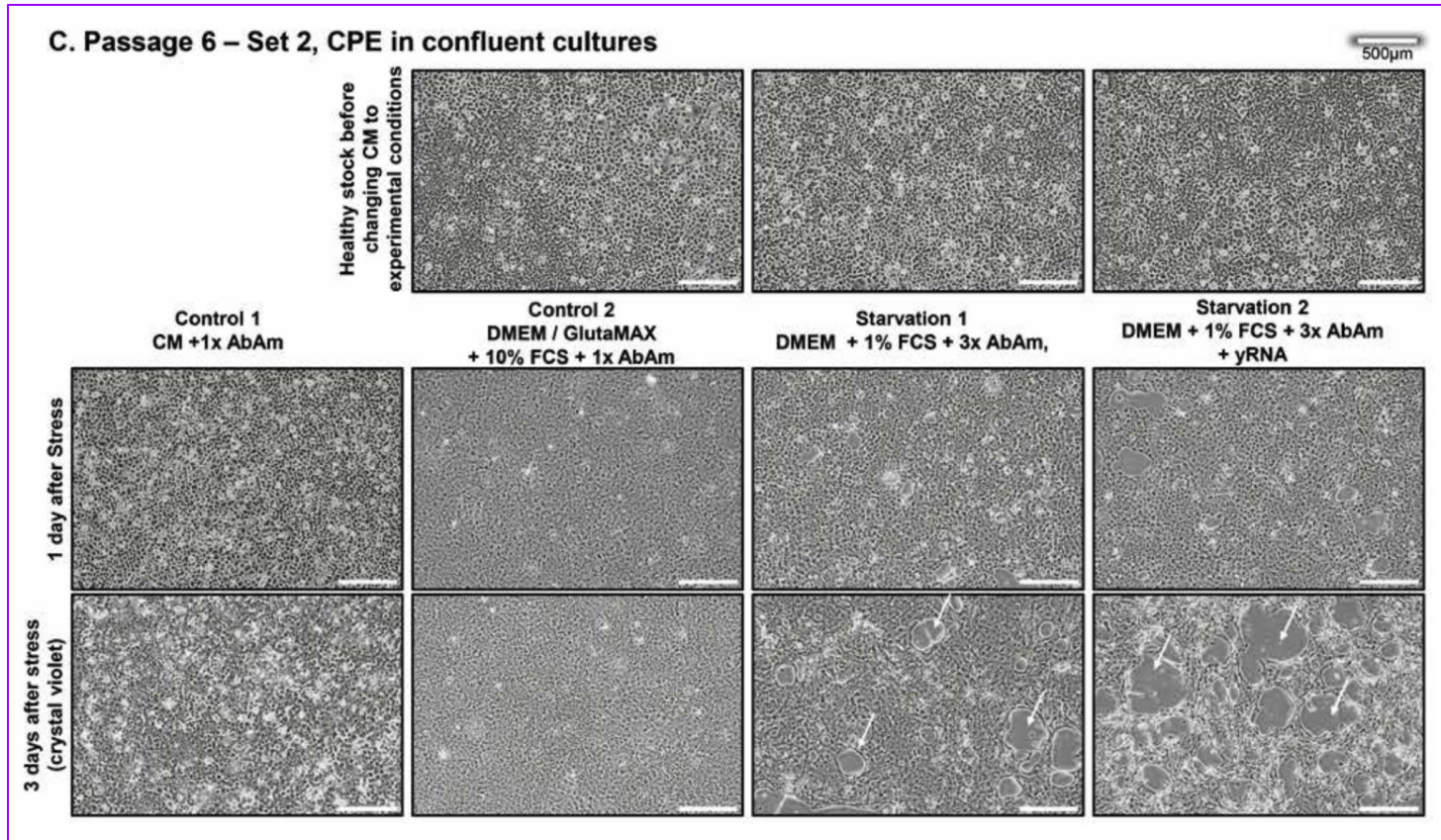
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ABSTRACT: Fetuses were collected from four Mexican free-tailed bats (*Tadarida brasiliensis mexicana*) and a fetal bat cell (FBC) line was established and tested for its ability to support the replication of the ERA vaccine strain of rabies virus. Cytopathic effects were detected in ERA virus-inoculated as well as uninoculated FBC's. Immunofluorescent antibody testing of uninoculated FBC's provided no evidence for the presence of rabies virus. However, mice inoculated intracranially with supernatant fluid from uninoculated FBC's died. Enzyme-linked immunosorbent assay and immunofluorescent antibody testing revealed rabies virus in the brains of these mice. Tests with a panel of monoclonal antibodies indicated that the isolate was the same as that isolated from Mexican free-tailed bats from the southwestern United States. We conclude that the fetuses from which the FBC line was derived had been infected in utero with rabies virus. We believe this may represent the first observation of prenatal transfer of rabies virus in naturally infected bats.

Key words: Rabies virus, Mexican free-tailed bat, *Tadarida brasiliensis mexicana*, in utero, prenatal infection, epidemiology, experimental study.

below). For example, it was possible that structures resembling knobs might be observed even when there was no gp120 present, i.e., false positives. On the other hand, any attempts to purify the virus would have caused additional loss of knobs.

clusters are hardly protrusions at all. We suggest that the spikes observed by negative-staining electron microscopy may be an artifact of the penetration of heavy metal stain between envelope proteins. Indeed, the term “spike” appears to have assumed a rather imprecise, possibly misleading definition, and might best be used with caution. In the work of Briggs et al. (6), the spikes on the surfaces of HIV virions protruded about 7.5 nm, which is not inconsistent with the height above the virion surface that we observe by AFM for the tufts of protein. On the other hand, spikes of envelope protein are described on the surfaces of HFV that extend 13.8 nm above the surface (69), nearly twice the length of those on HIV. The gp41-gp 120



Experimental Group Huh7

- Cell Culture Plate: 24 wells
- Cell Lines: Huh7
- DMEM: 0.5ml per well
- FBS: 2%
- Anti-Anti: 2% (1st gen), 1% (next gen)
- Trypsin: None

Experimental Group Vero E6

- Cell Culture Plate: 24 wells
- Cell Lines: Vero E6
- DMEM: 0.5ml per well
- FBS: 2%
- Anti-Anti: 2%(1st gen), 1% (next gen)
- Trypsin: None

Control Group Huh7

- Cell Culture Plate: 24 wells
- Cell Lines: Huh7
- DMEM: 0.5 ml per well
- FBS: 2%
- Anti-Anti: 1%
- Trypsin: None

Control Group Vero E6

- Cell Culture Plate: 24 wells
- Cell Lines: Vero E6
- DMEM: 0.5 ml per well
- FBS: 2%
- Anti-Anti: 1%
- Trypsin: None

CPE

How many wells demonstrated CPE from each group?

For control, cell grew well.

For the samples, only one CPE.

[Quoted text hidden]

Out of 48 wells, 24 Huh7 & 24 Vero E6, only 1 from each exhibited CPE!

4. MISINTERPRETATION OF ALLEGED “PATHOGENIC VIRUSES”.

Unusual amounts of copper have been found in puri-

¹⁸ Lauffer and Ross, *Jour. Am. Chem. Soc.*, 62: 3296, 1940; McFarlane and Kekwick, *Biochem. Jour.*, 32: 1607, 1938; Melchers, *et al.*, *Biol. Zentr.*, 60: 524, 1940.

¹⁹ Sharp, Taylor, Beard and Beard, *Jour. Biol. Chem.*, 142: 193, 1942.

²⁰ Shedlovsky and Smadel, *Jour. Exp. Med.*, 72: 511, 1940; Smadel, Pickels, Shedlovsky and Rivers, *ibid.*, 72: 523, 1940.

²¹ Polson, *Onderstepoort J. Vet. Sci.*, 16: 51, 1941.

²² Hoagland, *et al.*, *Jour. Exp. Med.*, 71: 737; 72: 139, 1940; 74: 69, 133, 1941; 76: 163, 1942.

²³ Taylor, Sharp, McLean, Beard, Beard, Dingle and Feller, *Jour. Immun.*, 48: 361, 1944.

²⁴ Knight, *Jour. Exp. Med.*, 80: 83, 1944.

Viruses have been especially easy to recognize through the diseases they produce, but we are now learning that other substances of similar particle size exist. These substances frequently complicate and even render impossible the purification of viruses. In fortunate instances they are less stable or sufficiently different in size to be separable by a selective procedure such as ultracentrifugation. But cases are be-

²⁵ Knight, *Jour. Am. Chem. Soc.*, 64: 2734, 1942.

²⁶ Avery, MacLeod and McCarty, *Jour. Exp. Med.*, 79: 137, 1944.

²⁷ Berry, *Arch. Path.*, 24: 533, 1937.

ing found where viruses have much the same size and stability as components of the healthy tissues involved. Mouse lungs infected with influenza²⁸ provide an example. Ultrafiltration or centrifugation of normal lungs yields a suspension of particles about 100 m μ in diameter. Infectiousness is found associated with particles of this size when infected lungs are similarly treated, and it has required much work to prove that the virus itself is not a far smaller entity adsorbed to "healthy" 100 m μ particles. Allantoic fluid from

from them will be superior to those made with crude tissue suspensions. As yet there is little understanding of the changes that take place in a virus particle during inactivation either by chemicals or radiation. Effective killed virus vaccines are made with formaldehyde, but the reaction is in fact unknown and we are in no position to say whether the essentially empirical procedures now employed in making vaccines are the most desirable ones. A knowledge of the absorption spectrum of purified viruses provides a similar sound

V. SUMMARY

Electron-microscopic study of purified preparations, derived from central nervous system tissue of mice and cotton rats by differential centrifugation, has shown that particles alike as to size and shape are obtained from both normal and poliomyelitic tissue. The total yield of nitrogenous material has been essentially the same from both normal and infected tissues.

Characteristic particles separated from both normal and infected tissues are approximately spherical and in the size range from 6 to 30 $m\mu$ in diameter. Aggregates of the particles are often obtained.

No intact rods or long filaments have been detected in any of the preparations, but some of the aggregations could easily be mistaken for such forms in unshadowed micrographs.

From a critical examination of our work, and that of others, we conclude that there is no evidence that a virus of the poliomyelitis group has ever been unequivocally identified on electron micrographs thus far published.

SUMMARY

Filaments morphologically identical to the so-called filamentous forms of influenza and fowl pest viruses were observed with the electron microscope in skin tissue fluid from a patient with pinta, a tropical treponematosi. Particles resembling those of influenza, poliomyelitis, and pox group viruses are seen in several fields. Evidence is presented that many of these appearances are artifacts originating from erythrocyte stromatolysis, whereas others are either crystals with rounded corners or what may be protein particles.

The work of a group of investigators in Paris has shown that virus-like particles morphologically indistinguishable from the known chicken tumor viruses can also be found in thin sections of tissues obtained from "normal" chickens, chicks, or chick embryos (3, 5). Similar particles were seen in sections of tissue cultures originating from "normal" chick tissues (15). The present report confirms this previous information. It is interesting to note that in the present case no deliberate effort was made to detect virus particles within normal chick tissues. The fact that the particles were incidentally found even within the limited tissue material which can be surveyed in thin sections seems to indicate that an enormous number of such particles was present within this aorta and this liver.

SUMMARY—A small virus-like particle was observed by means of thin-section electron microscopy in 9 of 24 (37%) human breast cancer biopsies, in density-gradient purified milk specimens from 4 of 7 (57%) breast cancer patients, and in 7 of 43 (16%) normal women examined to date. The ultrastructural morphology, which reveals a particle of approximately 30 m μ in diameter with an inner electron-dense nucleoid and a double outer membrane, appeared identical in all three materials.—J Nat Cancer Inst 40: 1359–1373, 1968.

if little endogenous agent is produced. At least these findings suggest that FBS is widely contaminated with virus-like particles of unknown biologic properties. The possibility of such a contaminant adds an uncontrolled variable to studies attempting to detect and propagate a human tumor virus whose properties are likewise unknown.

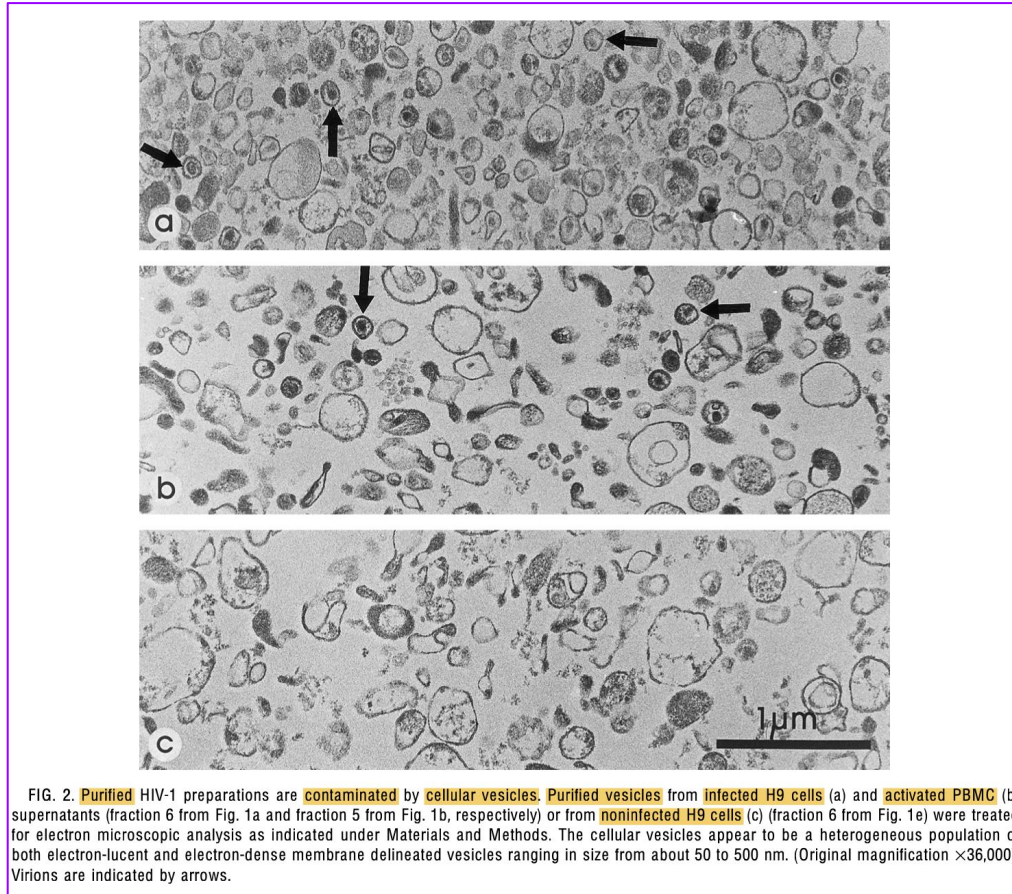
MULTIVESICULAR BODIES were first described by Palay and Palade in neurons in 1955 (1). This study and others (1–5) concentrated on neurons, but multivesicular bodies are normal constituents of other cell types (6), particularly secretory cells (7–9). Their origin is not completely clear, but there is convincing evidence that they are involved in the lysosome system of cells (7, 10). **The vesicles of multivesicular bodies have been confused with viruses and, with some justification, have in the past been called “virus-like particles”** (11). However, Haguenu (12) states:

4.7. (b).

["Virus-like" particles in FBS, 1975]

More recently, Benz and Moses (14) described "virus-like" particles in all samples of FBS examined and warned about the possibility that ". . . such a contaminant adds an uncontrolled variable to studies attempting to detect and propagate a human tumor virus whose properties are likewise unknown." Our unpublished results of 1967 confirm their findings and the figure 6 is comparable to their figure 1. It also

Viral particles have been demonstrated by electron microscopy in lymph nodes from patients with acquired immune deficiency syndrome AIDS-related persistent generalized lymphadenopathy (PGL) syndrome. Immunohistochemical and *in situ* hybridization studies have identified these viruses as the human immunodeficiency virus (HIV). In this study, we examined 20 PGL lymph nodes and found viral particles in 18 cases. Immunohistochemical studies on these cases revealed positive staining for the HIV core protein P24 within germinal centers of secondary follicles. In addition we found viral particles, morphologically indistinguishable from those observed in PGL lymph nodes, in 13 of 15 non-HIV related reactive lymph nodes. Immunohistochemical staining of these lymph nodes for the P24 core protein was negative. None of the patients in this group had risk factors for developing AIDS and none exhibited clinical evidence of immune deficiency. We conclude that the viral particles observed in PGL lymph nodes are most likely HIV, but similar particles can be seen in reactive lymph nodes not associated with HIV infection. The discrete localization of these particles within germinal centers has been observed for other viruses and immune complexes and a possible mechanism of this antigen deposition is discussed. HUM PATHOL 19:545-549, 1988.



4.10. (a).

[Clathrin coating, not "Virus-spikes", 2009]

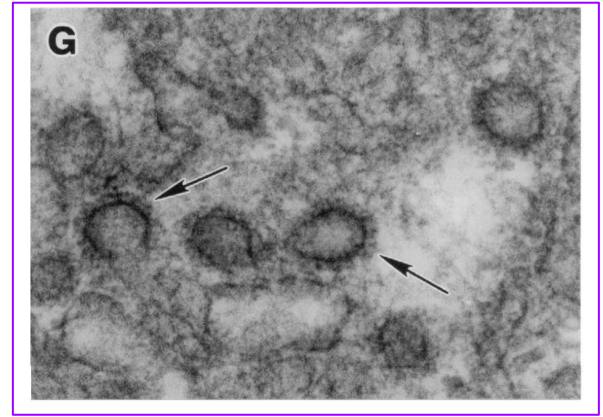
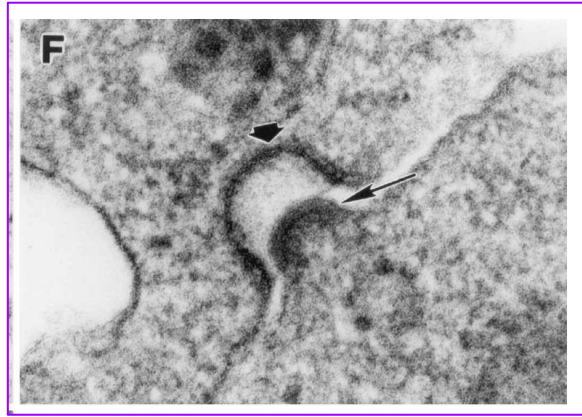


FIG. E, F, & G *Pinocytotic vesicles (E) and coated pits/vesicles are notorious for masquerading as budding virions. In this instance, an HIV particle (arrow) budding outward from the plasma membrane has induced the formation of a coated endosome (arrowhead) on an adjacent cell (F). The clathrin coating on endosomes resembles viral spikes (G, arrows). When seen in degenerating cells, Golgi vesicles can resemble free viral particles. (E) $\times 66,000$, (F) $\times 137,000$ (G) $\times 110,000$.*

4.10. (b).

[Lysosomes, not "Viruses", 2009]

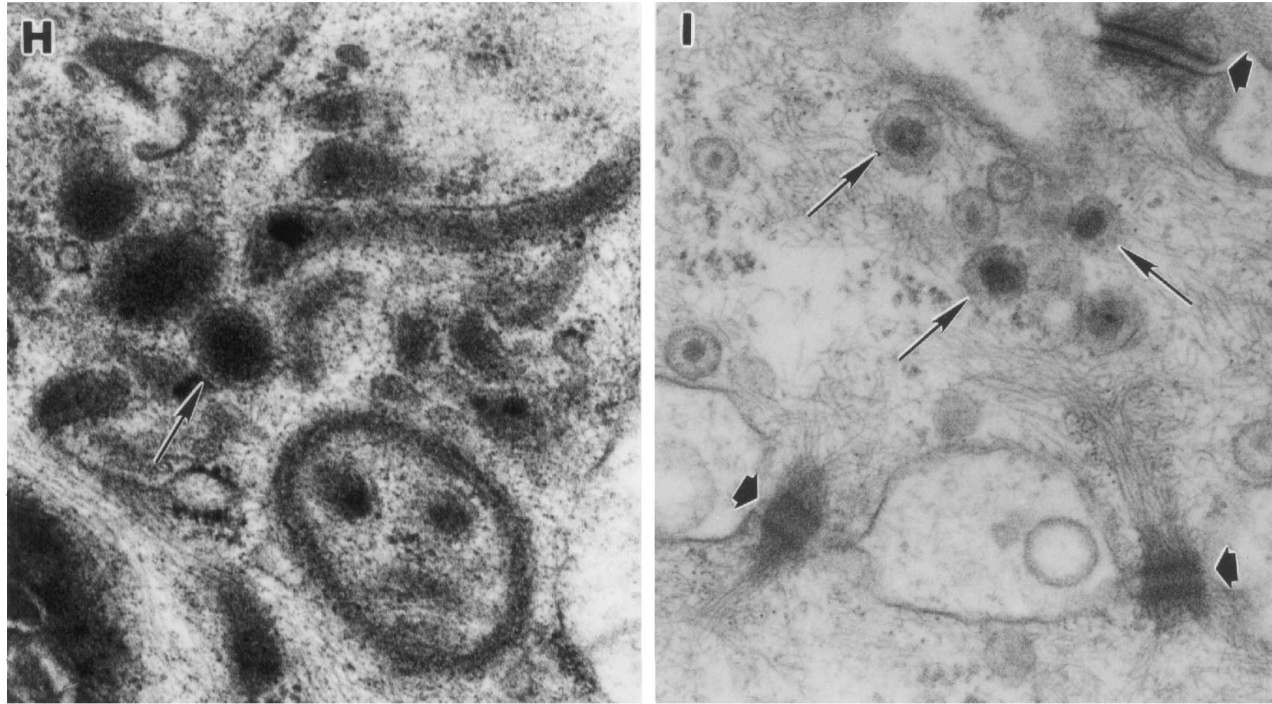


FIG. H & I *Lysosomes can resemble dense core neuroendocrine granules, as well as mimicking viral particles (H, arrow). Lysosomes (arrows) are often located near the plasma membrane of keratinocytes and because of the relatively large halo around its dense core can be mistaken for herpesviruses (I). Note the well-formed desmosomes (arrowheads) (H) $\times 58,000$, (I) $\times 51,000$.*

4.10. (c).

[Inclusions, not "Viruses", 2009]

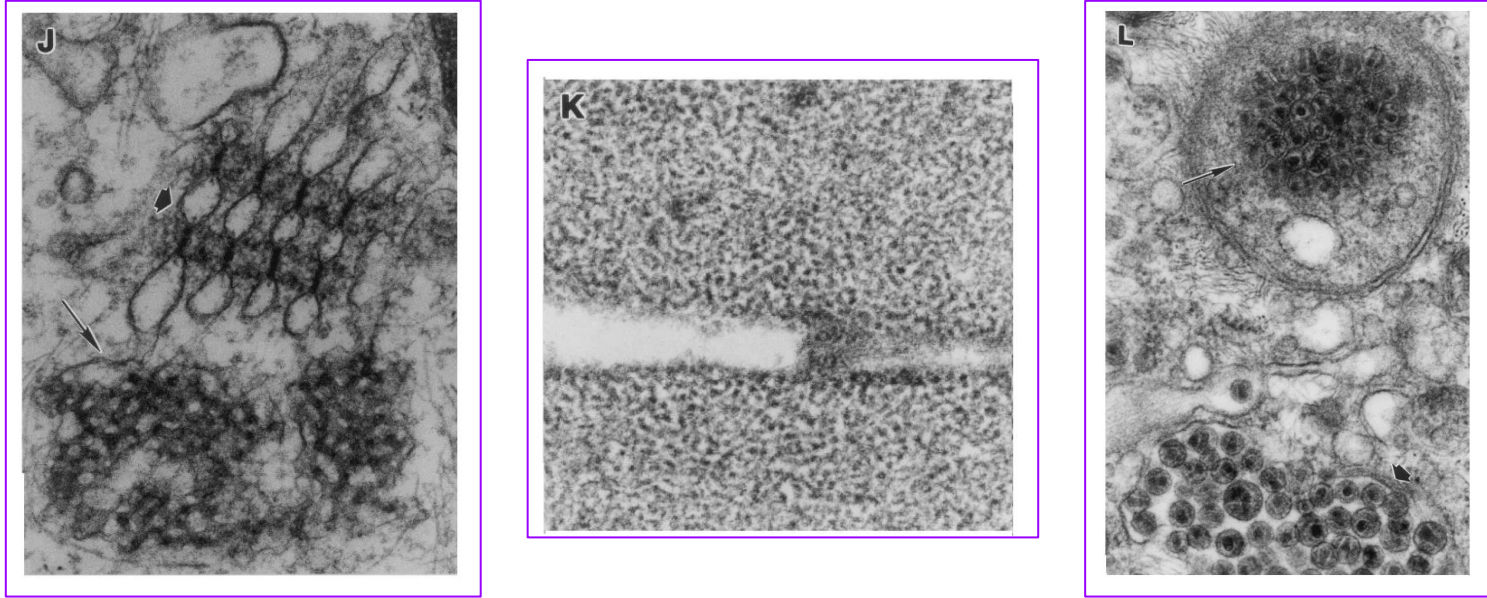


FIG. J & K *IFN-alpha tubuloreticular inclusions (J, arrow) and annulate lamellae (arrowhead) can resemble viruses. The chromatin (K) of some necrotic cells can resemble paramyxovirus. (J) $\times 110,000$, (K) $\times 66,000$.*

FIG. L *HIV replicate at the surface of cytoplasmic vacuoles and plasma membranes. However, when a vacuole is cut tangentially, the unit membrane may be obscured and the virions can appear to be free in the cytosol (arrow). Note that there is another intracytoplasmic vacuole with virions that has a distinct unit membrane. (L) $\times 58,000$.*

4.10. (d).

[Inclusions, not "Viruses", 2009]

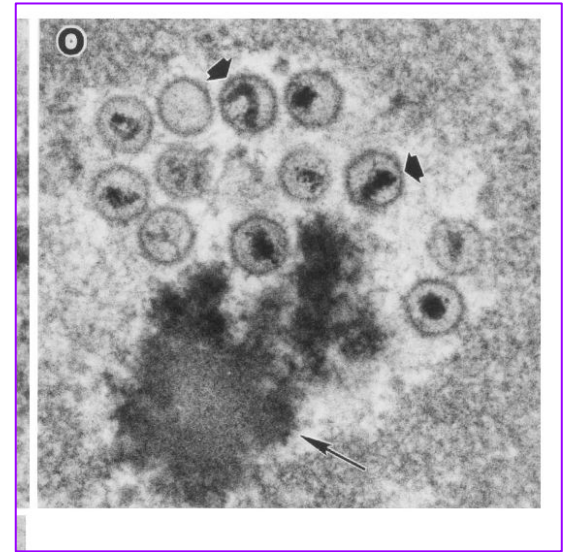
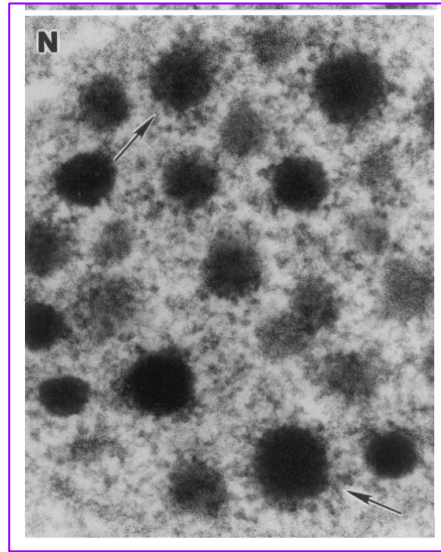
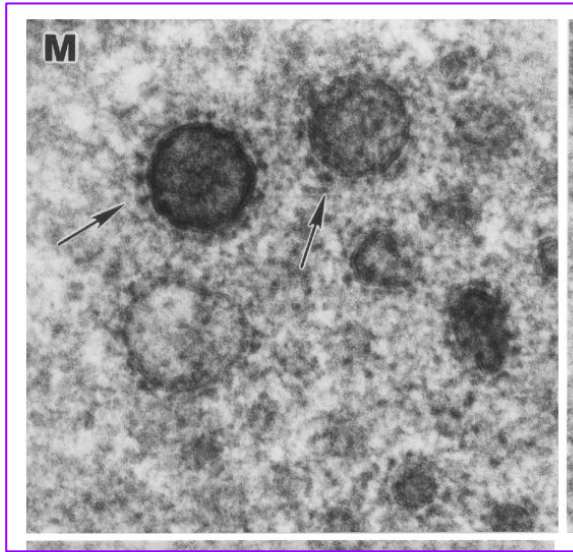


FIG. M, N & O *There are many types of intranuclear inclusions. Some can resemble viruses with spikes (M, N, arrows) while others are truly viral nucleocapsids, like CMV (O, arrows). When DNA (arrow) is present within the nucleoid (arrowhead) it can vary considerably in appearance. M \times 105,000, N & O \times 110,000.*

4.10. (e).

[Cell contents, not "Viruses", 2009]

6. Nuclear pores and annulate lamella can resemble spherical viral particles, especially when cut *en face* (Figures C, D).
 7. Pinocytic vesicles "bud inward" (Figure E) while virions "bud outward" (Figure F) from the plasma membrane. Calthrin-coated vesicles go through a budding-like process and are covered with bristles that can resemble viral spikes (Figure G).
 8. Primary lysosomes and dense core granules can be mistaken for many things, including viral particles, especially herpesviruses (Figure H). Squamous cells have lysosomes, located near their plasma membranes, that have dense cores and a wide halo and are often confused with virions (Figure I).
 9. Multivesicular bodies (MVB), a component of the Golgi system, contain small vesicles that can resemble virions and can have an electron-dense area of its membrane that can be misinterpreted as an area of viral fusion.
 10. Tubuloreticular inclusions (TRI), which form in the RER in response to acid-labile, interferon-alpha, can resemble paramyxovirus (Figure J). Presumably for lack of knowledge of the literature, the TRI is "rediscovered" every few years.
 11. The "worm-like" chromatin in degenerating/necrotic cells can be misinterpreted as a specific change or confused with paramyxovirus (Figure K).
 12. Electron-dense chromatin/interchromatin granules can resemble many things, including virions; however, they vary in size, are often surrounded by an electron-lucent area, and are relatively simple in appearance as compared to virions.
 13. Virions within cytoplasmic compartments, such as in Golgi vacuoles or RER can appear to be free in the cytoplasm of poorly preserved or necrotic cells or when there is tangential sectioning (Figure L).
 14. Intranuclear inclusions vary considerably in appearance and can even have viral spike-like structures (Figures M, N), something that would not normally appear on immature nucleoids (Figure O).
 15. Elongated forms of mycoplasma can resemble villous cell processes.
 16. Shedding of portions of the plasma membrane in the form of whorl-like structures can resemble budding viral particles.
- Needless to say, the literature is rife with misinterpretations. Some of these have been pointed out, while others languish. It is important to remember that most scientists are not familiar with interpreting electron micrographs and take the authors' interpretations at face value. It is the responsibility of electron microscopists to reduce mistakes and maintain credibility. Usually, journal editors are conscientious

be viewed using a TEM. With this method the background is stained and particles, including intact virions are left unstained, therefore outer details of the virus are visualized against the electron-dense background. Care must be taken with interpretation since the sample contains other cellular debris that can be in the size range of a virus. Notice the cellular debris in the negative staining sample in Figure 1a; careful interpretation must distinguish between virus and other cellular elements, such as broken down

September 2014

2.1.2 Electron Microscopy for Diagnostic Virology - 1

cellular membranes. Additional information regarding negative staining in diagnostic virology can be found in Goldsmith & Miller (2009).

We read with interest the Correspondence by Zsuzsanna Varga and colleagues¹ on the possible infection of endothelial cells by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using electron microscopic (EM) images as evidence. However, we believe the EM images in the Correspondence do not show coronavirus particles but instead show cross-sections of the rough endoplasmic reticulum (RER). These spherical structures are surrounded by dark dots, which might have been interpreted as spikes on coronavirus particles but are instead ribosomes. The purported particles are free within the cytoplasm, whereas within a coronavirus-infected cell, accumulations of virus particles would be found in membrane-bound

seen in the interior of these structures as would be found with coronavirus particles (figure).

Just recently, there have been two additional reports^{3,4} in which structures that can normally be found in the cytoplasm of a cell have been misinterpreted as viral particles.⁵

EM can be a powerful tool to show evidence of infection by a virus, but care must be taken when interpreting cytoplasmic structures to correctly identify virus particles.

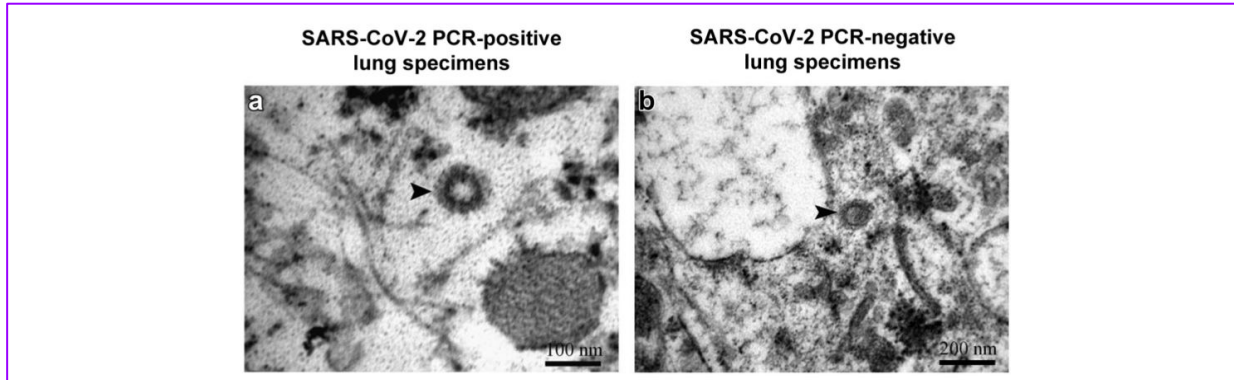
We declare no competing interests. The findings and conclusions are those of the authors and do not necessarily represent the position of the US Centers for Disease Control and Prevention.

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Recognition of this pitfall of “viral-like particles” actually dates back to the 1970s, when the potential for mistakenly assuming that normal cellular components, such as phagocytic vacuoles, microvesicular bodies, or extracellular breakdown products, could represent viral particles was emphasized after a proliferation of studies claiming to have found ultrastructural viral particles within different types of cancer cells and fluids (23). Thus, we would like to echo the CDC (9) and earlier authors’ observations (15,23) and issue a note of

injury, proteinuria, and hematuria. Recent publications in *Kidney International* used electron microscopy (EM) to detect the virus in autopsy or biopsy specimens of the kidney.^{1,2}

Most of the published images depicting the suspected virus are very similar, if not identical, to multivesicular bodies (MVBs). MVBs have been well-known since the 1960s and their appearance and occurrence is detailed in the classic monograph of Feroze Ghadially;³ however, their exact significance and function is unclear. We suspect that the EM images of SARS-CoV-2 published to date are in fact MVBs.



SARS-CoV-2 PCR-negative kidney specimens from COVID-19 patient

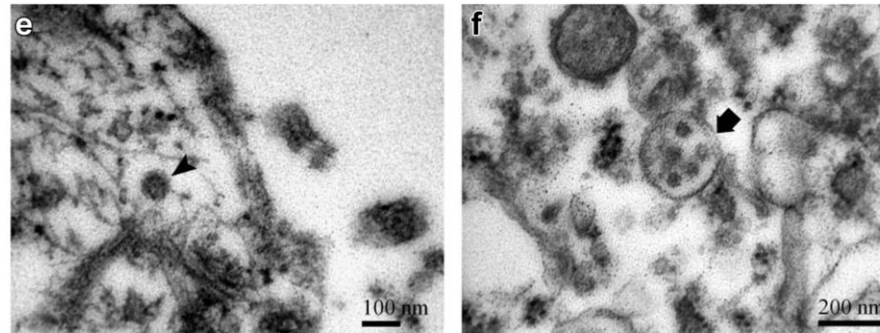
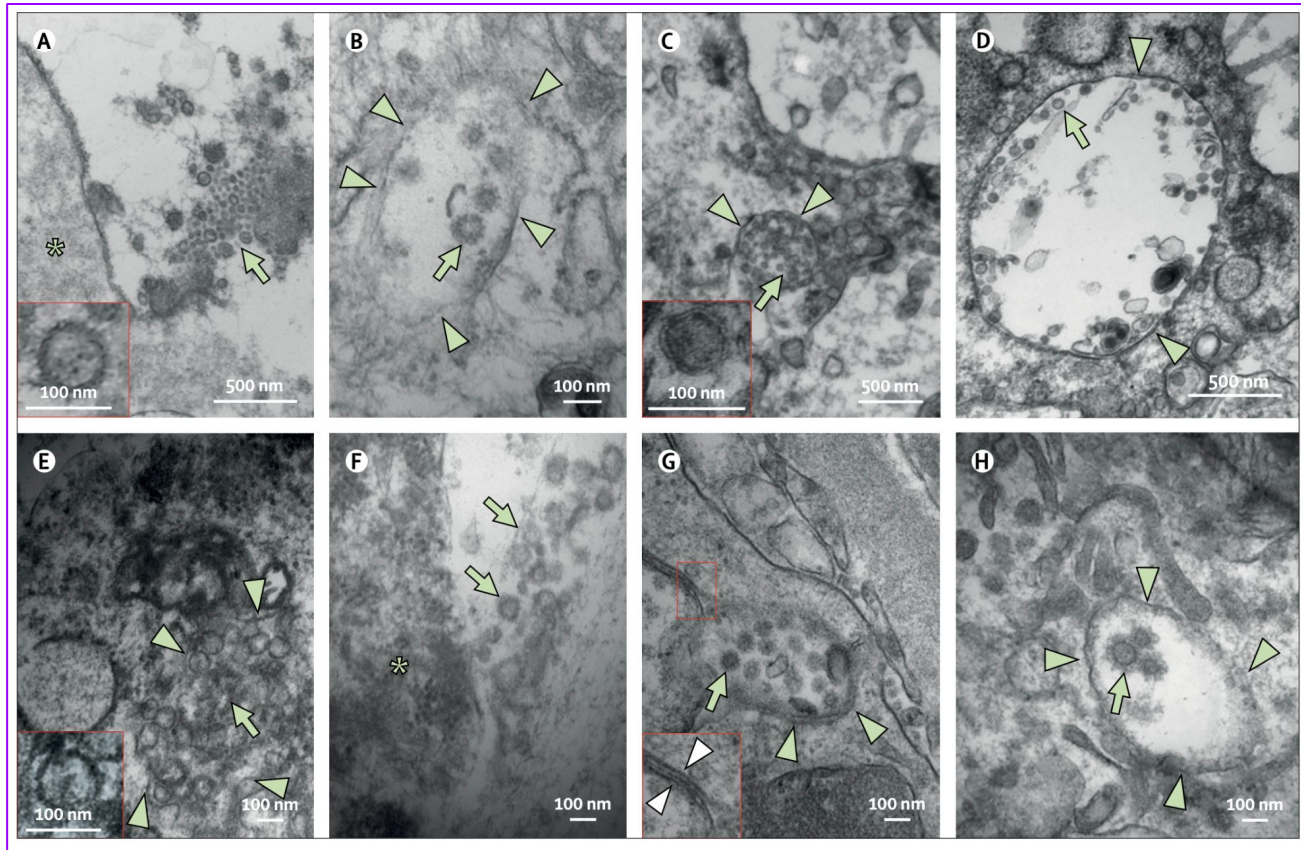


Figure 1. Individual vesicle with electron-dense coat (arrowhead) located freely in the cytosol of endothelial cell in lung with positive reverse-transcriptase polymerase chain reaction (RT-PCR) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA (a) and in lung with negative RT-PCR for SARS-CoV-2 RNA (b). Note similar morphology of the 2 structures in images (a) and (b), which could be virus or coated vesicle. (Continued)

4.16. (a).

[Vesicles, not "SARS-CoV-2", 2020]



4.16. (b).

[Vesicles, not “SARS-CoV-2”, 2020]

Figure 5: Ultrastructural features in fatal COVID-19 infections

Ultrastructural finding of viral particles in tracheal epithelial cells (A and B) in patient 13, lung pneumocytes (C and D) in patient 13, enterocytes (E and F) in patient 13, and kidney endothelial cells (G) in patient 8 and proximal tubular epithelial cells (H) in patient 13. Viral particles (indicated by green arrows) were observed either outside cells (A and F) in close proximity to the cell membrane or inside the cells (B, C, D, E, G, and H) in aggregates confined within vesicles (indicated by green arrowheads). Some of the particles were associated with double membranes (indicated by white arrowheads) resembling double membrane vesicles. Asterisks in (A) and (F) mark the cells adjacent to the viral particles in the extracellular space.

In the article by Farkash *et al.*,⁸ the electron microscopic images in their Figure 3, A–C do not demonstrate coronaviruses. Rather, the structures described as virus are clathrin-coated vesicles (CCVs), normal subcellular organelles involved in intracellular transport. Figure 3A⁸ is a

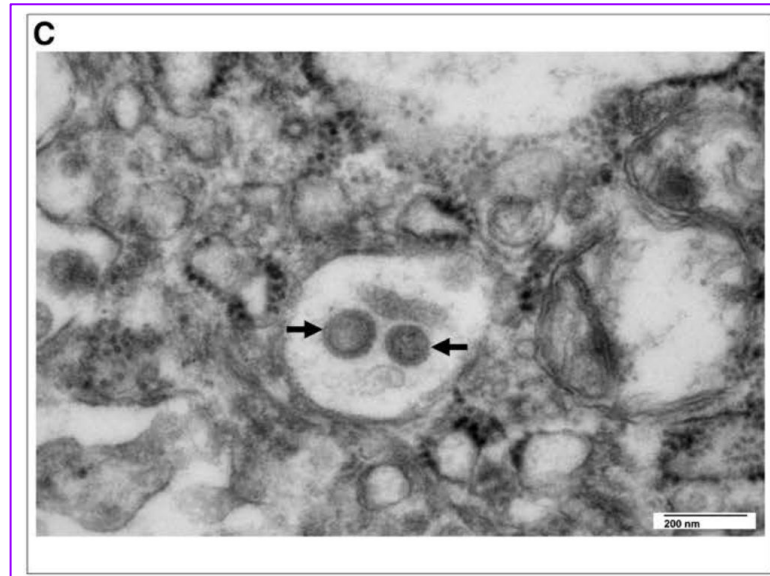


FIGURE 1. Histopathologic features of the placenta included histiocytic intervillitis (arrows, **A**, hematoxylin and eosin, $\times 200$ magnification), immunohistochemical staining for SARS-CoV nucleocapsid protein in the cytoplasm of the syncytiotrophoblastic cells (**B**, immunoperoxidase, $\times 200$ magnification), and viral-like particles (arrows) in membrane-bound cisternal spaces in the syncytiotrophoblastic cells (**C**, transmission electron microscopy, $\times 40,000$ magnification).

Why misinterpretation of electron micrographs in SARS-CoV-2-infected tissue goes viral

With interest we follow the publications that show the presence of putative severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by electron microscopy (EM) in patient tissues and the debate about these results, which should have sufficiently raised attention to their correct interpretation.^{1,2}

Nevertheless, ultrastructural details in autopsy tissues have been misinterpreted as coronavirus particles in recent papers. Bradley and

internalize cargos from the extracellular space.² Coated vesicles and coated pits derive their name from the external scaffold coat composed of clathrin triskelions that decorate the surface of the structure. In transmission electron micrographs in which tissue-thin sections are stained with uranyl acetate and lead citrate, coated vesicles have an electron-dense studded surface that appears identical to the “corona” comprising the spike protein that decorates all coronaviruses, including SARS-CoV-2 virions. It is this studded surface or corona that gives the genus *Betacoronaviridae* its characteristic morphology and name.

Hopfer H, Herzig MC, Gosert R, Menter T, Hench J, Tzankov A, Hirsch HH & Miller SE.

(2021) *Histopathology* 78, 358–370. <https://doi.org/10.1111/his.14264>

Hunting coronavirus by transmission electron microscopy – a guide to SARS-CoV-2-associated ultrastructural pathology in COVID-19 tissues

Abstract: Transmission electron microscopy has become a valuable tool to investigate tissues of COVID-19 patients because it allows visualisation of SARS-CoV-2, but the ‘virus-like particles’ described in several organs have been highly contested. Because most electron microscopists in pathology are not accustomed to analysing viral particles and subcellular structures, our review aims to discuss the ultrastructural changes associated with SARS-CoV-2 infection and COVID-19 with respect to pathology, virology and electron microscopy. Using micrographs from infected cell cultures and autopsy tissues, we show how coronavirus replication affects ultrastructure and put the morphological findings in the context of viral replication, which induces extensive

remodelling of the intracellular membrane systems. Virions assemble by budding into the endoplasmic reticulum–Golgi intermediate complex and are characterised by electron-dense dots of cross-sections of the nucleocapsid inside the viral particles. Physiological mimickers such as multivesicular bodies or coated vesicles serve as perfect decoys. Compared to other *in-situ* techniques, transmission electron microscopy is the only method to visualise assembled virions in tissues, and will be required to prove SARS-CoV-2 replication outside the respiratory tract. In practice, documenting in tissues the characteristic features seen in infected cell cultures seems to be much more difficult than anticipated. In our view, the hunt for coronavirus by transmission electron microscopy is still on.

The severe acute respiratory syndrome coronavirus 2 pandemic has infected millions of individuals in the United States and caused hundreds of thousands of deaths. Direct infection of extrapulmonary tissues has been postulated, and using sensitive techniques, viral RNA has been detected in multiple organs in the body, including the kidney. However, direct infection of tissues outside of the lung has been more challenging to demonstrate. This has been in part due to misinterpretation of electron microscopy studies. In this perspective, we will discuss what is known about coronavirus infection, some of the basic ultrastructural cell biology that has been confused for coronavirus infection of cells, and rigorous criteria that should be used when identifying pathogens by electron microscopy. (*Am J Pathol* 2021, 191: 222–227; <https://doi.org/10.1016/j.ajpath.2020.11.003>)

4.23. (a).

[Subcellular structures, not “SARS-CoV-2”, 2021]

Structures Commonly Misidentified as Coronaviruses

We performed a literature search for reports published during March 1–November 30, 2020, that used EM to identify coronavirus directly in patient specimens. We used the keywords ultrastructure or electron microscopy in conjunction with COVID-19, SARS-CoV-2, or coronavirus when searching Google Scholar, PubMed, MEDLINE, Web of Science, and Scopus. We identified 27 reports with EM findings. Four of these reports and 1 letter to the editor included correctly identified coronavirus (30–34). The other 23 articles revealed a pattern of subcellular structures misidentified as virus (Table), including clathrin-coated and coatomer-coated vesicles (CCVs; 48%), multivesicular bodies (MVBs; 26%), circular cross-sections through vesiculated RER (19%), spherical invaginations of RER (4%), and other nonviral structures (30%). Figure 2 shows an overview of these subcellular components observed within autopsy tissues.

4.23. (b).

[Subcellular structures, not “SARS-CoV-2”, 2021]

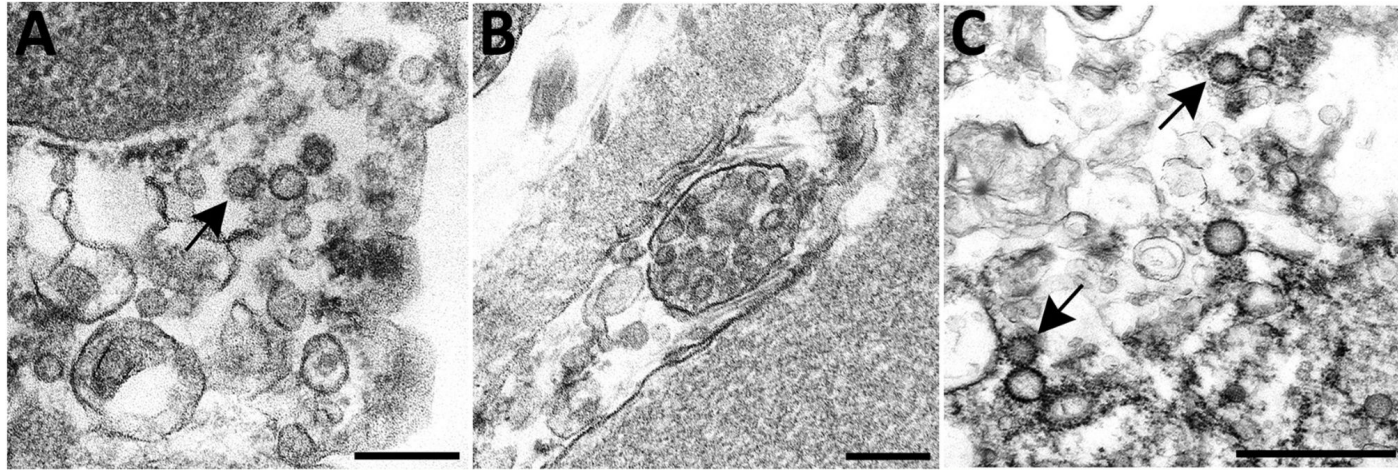


Figure 2. Overview of differential ultrastructural features of subcellular structures commonly misidentified as coronaviruses; all were prepared by thin section from formalin-fixed autopsy specimens. A) Clathrin-coated vesicles (CCVs), circular vesicles with a fringe of clathrin protein (arrow), in the cell cytoplasm range in size from 60 nm–100 nm. Differentiation: clathrin surrounding the vesicle may be misinterpreted as viral spikes, however, CCVs are free in the cell cytoplasm, and clathrin is in direct contact with the cytoplasm. Intracellular coronaviruses are found within membrane-bound vacuoles, and spikes, if visible, are in contact with the vacuolar contents. CCVs lack the internal black dots that signify cross sections through the viral nucleocapsid. Scale bar indicates 200 nm. B) Multivesicular body (MVB), a collection of membrane-bound roughly spherical vesicles formed by the inward budding of an endosomal membrane. Differentiation: MVBs may be confused with a vacuolar accumulation of coronavirus particles. Vesicles within multivesicular bodies do not have internal black dots that signify cross sections through the viral nucleocapsid. Scale bar indicates 200 nm. C) Circular cross sections through rough endoplasmic reticulum (RER) (arrows) found free within the cytoplasm. Differentiation: ribosomes along the endoplasmic reticulum may be confused with viral spikes. Ribosomes of vesiculating RER are in direct contact with the cell cytoplasm, unlike coronavirus spikes, which would be in contact with vacuolar contents. Vesiculating RER lacks cross sections through the viral nucleocapsid. Scale bar indicates 1 μ m.

4.23. (c).

[Subcellular structures, not “SARS-CoV-2”, 2021]

of the virus. Consequently, several articles have erroneously described the identification of coronavirus particles by EM in the lung (1-6), kidney (6-13; B. Diao et al., unpub. data, <https://doi.org/10.1101/2020.03.04.20031120>), heart (14,15), brain (16), liver (17), intestine (6,18), skin (19), and placenta (20-22) (Table). However, most of the presumed virus or virus-like particles shown in all of these reports either represent normal subcellular organelles previously demonstrated in cells (23) or, otherwise, lack sufficient ultrastructure and morphologic features to be conclusively identified as coronavirus. Since early May 2020, letters to the editors of several journals have refuted these descriptions (24-30), yet the misidentification of coronavirus particles continues. It is essential for our collective understanding of COVID-19 clinical pathology and pathogenesis as well as the field of diagnostic EM that these misidentifications of SARS-CoV-2 particles be addressed.

*Article*

Just Seeing Is Not Enough for Believing: Immunolabelling as Indisputable Proof of SARS-CoV-2 Virions in Infected Tissue

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Abstract: Background: There is increasing evidence that identification of SARS-CoV-2 virions by transmission electron microscopy could be misleading due to the similar morphology of virions and ubiquitous cell structures. This study thus aimed to establish methods for indisputable proof of the

4.25. IMPORTANT NOTE

To prove the existence of any physical entity, two criteria has to be fulfilled at minimum, which are, **(i) the unique morphology** of that entity, and **(ii) the unique traits** of that entity. To prove the existence of a *hypothesised “pathogenic virus”*, at minimum, one has to **(i) prove the presence of an unique morphology** of a *hypothesised “pathogenic virus”* and then **(ii) prove** that the isolated entity which is hypothesised to be “pathogenic virus” **can cause dis-ease** when **inoculated through natural ways** onto the healthy subjects, with valid control experiments.

Though many studies were presented in this section about the misinterpretations of the alleged “pathogenic viruses”, **no one has ever isolated these photographed entities and proved their pathogenicity, nor proved the other hypothesised unique traits**. For example, in one of the foundational papers on the alleged “SARS-CoV-2 virus”, it is stated that, “...*our study does not fulfill Koch’s postulates...*” [[Ref. 5.1.](#)].

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The papers that has been published till date for the existence of Any Alleged "pathogenic virus", are Not based on *The Scientific Method* and *Logic!*

- Germ "Theory" Skeptics

PSEUDOSCIENTIFIC VIROLOGY - A BRIEF SUMMARY

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