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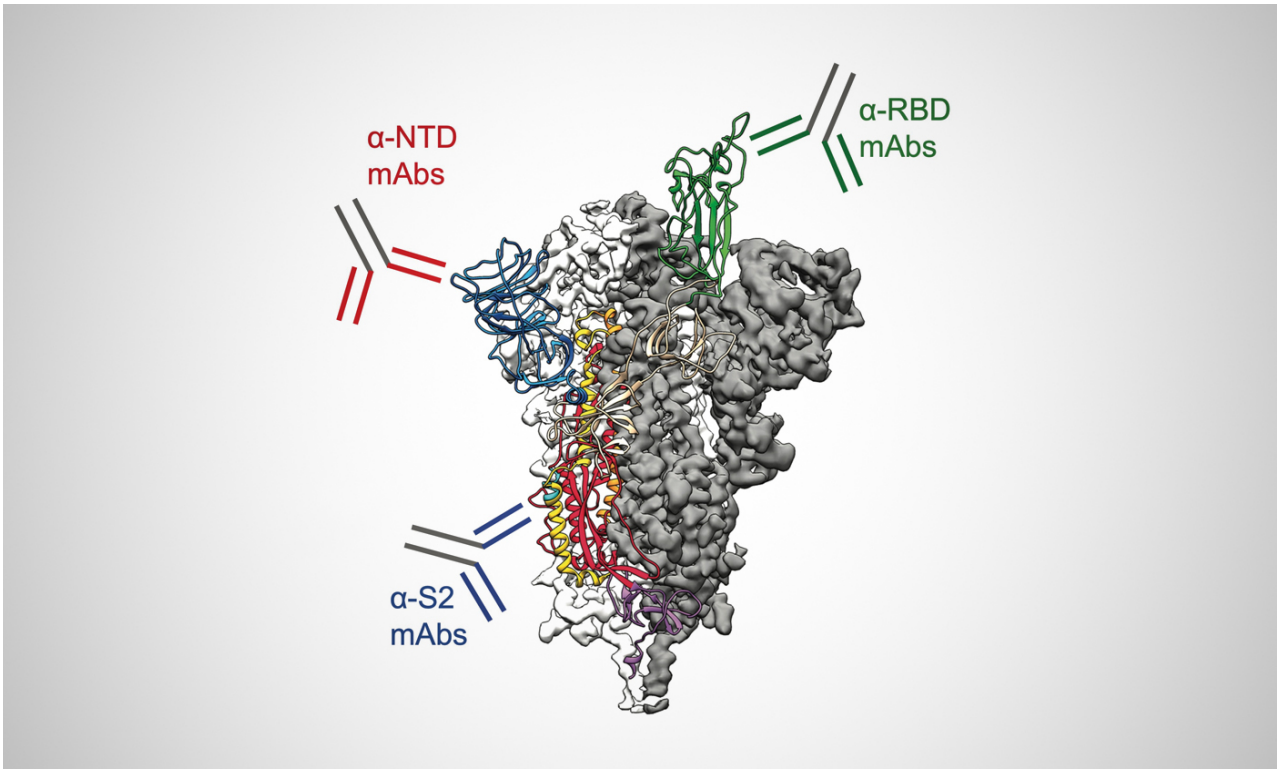
Exposing the lies of Germ Theory and virology using their own sources.

The Spike Protein



I often get asked many questions regarding the so-called “coronavirus” spike protein, such as whether or not it actually exists and whether it is actually in the vaccine. Does this particle hold any biological relevance whatsoever or is it just another in a long line of illustrations used to generate fear? If anyone is unaware at this point, the spike protein is said to be the 9-12 nm protrusions seen in TEM images of the “virus” which gives it the spiked or “corona” appearance around

the outside edges. According to mythology, these proteins allow the "virus" to penetrate the host cell and begin the infection process. The spike protein has risen to prominence due to the continued propaganda around the **mRNA vaccines < https://viroliegy.com/2021/11/07/the-uncharted-dangers-of-mrna-vaccines/>** which are claimed to produce the spike in the body once injected. This is said to trigger an immune response which teaches the body how to prepare an army of antibodies to fight off any sneaky stealh variant…or not. It depends on the variant, the manufacturer, the dose, the schedule, the disease outcome, etc.

According to the WHO:

> "Evidence on vaccine effectiveness (VE) against symptomatic illness and severe disease is expected to become available **only when variant-updated vaccines have been introduced into broader use."**

> "Currently available COVID-19 vaccines are based on the index virus (also referred to as the ancestral strain); however, **there has been continuous and substantial SARS CoV-2 viral evolution, particularly in the spike (S) protein.** These genomic changes in the virus have resulted in several VOC that have circulated in waves, **with varying degrees of immune evasion, some of which have resulted in lower VE of existing COVID-19 vaccines compared to the initial VE against the index virus.** The magnitude of the reduction in VE **varies by product, schedule, disease outcome, VOC and time since last dose.** From January to June 2022, the dominant SARS-CoV-2 variant globally has been the Omicron variant, with emergence of additional sub-lineages (1). The Omicron variant is the most antigenically distinct variant from the index virus and has exhibited the highest degree of immune evasion to current COVID-19 vaccines, compared to the index

virus. Current vaccines continue to perform well in preventing severe disease and death due to Omicron, particularly with the use of a booster dose(s). **However, protection against infection and symptomatic illness due to the Omicron variant is lower than other variants and declines rapidly, even after a third (booster) dose. Those at highest risk for severe disease, hospitalization, and death remain older persons, those with comorbidities and immunocompromising conditions, and other vulnerable populations as described in the WHO Prioritization Roadmap (2).”**

https://www.who.int/news/item/17-06-2022-interim-statement-on-decision-making-considerations-for-the-use-of-variant-updated-covid-19-vaccines < https://www.who.int/news/item/17-06-2022-interim-statement-on-decision-making-considerations-for-the-use-of-variant-updated-covid-19-vaccines>

The WHO states that the failure of the vaccines is due to the continual and rapid changes to the spike protein as it evolves and evades the immune systems defenses. This amazing super power has left us exactly in the same state we were in before the use of the toxic jabs over two years ago: the “virus” is still running rampant, the 99% of the population that were never at risk remain not at risk, and those who were at an increased risk of disease remain so. However, now we have many who are needlessly being injured by a vaccine never proven to be safe and effective with no data on long-term safety. It is clear that there was never any protection offered by the vaccine. The story created around the way the theoretical spike protein supposedly works is nothing but pure fiction attributed to an unobservable process said to occur inside the body after injection.

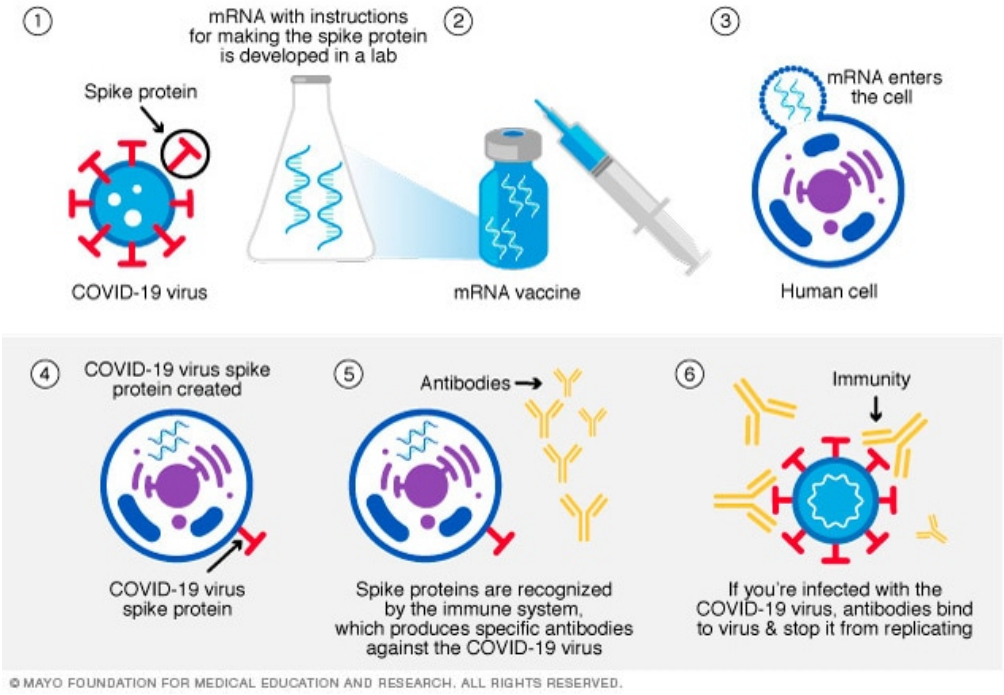
According to the CDC:

"To trigger an immune response, many vaccines put a weakened or inactivated germ into our bodies. Not mRNA vaccines. **Instead, mRNA vaccines use mRNA created in a laboratory to teach our cells how to make a protein—or even just a piece of a protein—that triggers an immune response inside our bodies. That immune response, which produces antibodies, is what helps protect us from getting sick from that germ in the future.**

1. First, mRNA COVID-19 vaccines are given in the upper arm muscle. **After vaccination, the mRNA will enter the muscle cells. Once inside, they use the cells' machinery to produce a harmless piece of what is called the spike protein.** The spike protein is found on the surface of the virus that causes COVID-19. **After the protein piece is made, our cells break down the mRNA and remove it.**
2. **Next, our cells display the spike protein piece on their surface. Our immune system recognizes that the protein does not belong there.** This triggers our immune system to produce antibodies and activate other immune cells to fight off what it thinks is an infection. This is **what your body might do** if you got sick with COVID-19.
3. **At the end of the process, our bodies have learned how to help protect against future infection with the virus that causes COVID-19.** The benefit is that people get this protection from a vaccine, without ever having to risk the potentially serious consequences of getting sick with COVID-19. **Any side effects from getting the vaccine are normal signs the body is building protection."**

https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/mRNA.html?s_cid=11344:how%20does%20mrna%20vaccine%20work:sem.ga:p:RG:GM:gen:PTN:FY21 < https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/mRNA.html?

s_cid=11344:how%20does%20mrna%20vaccine%20work:sem.ga:p:RG:GM:gen:PTN:FY21>

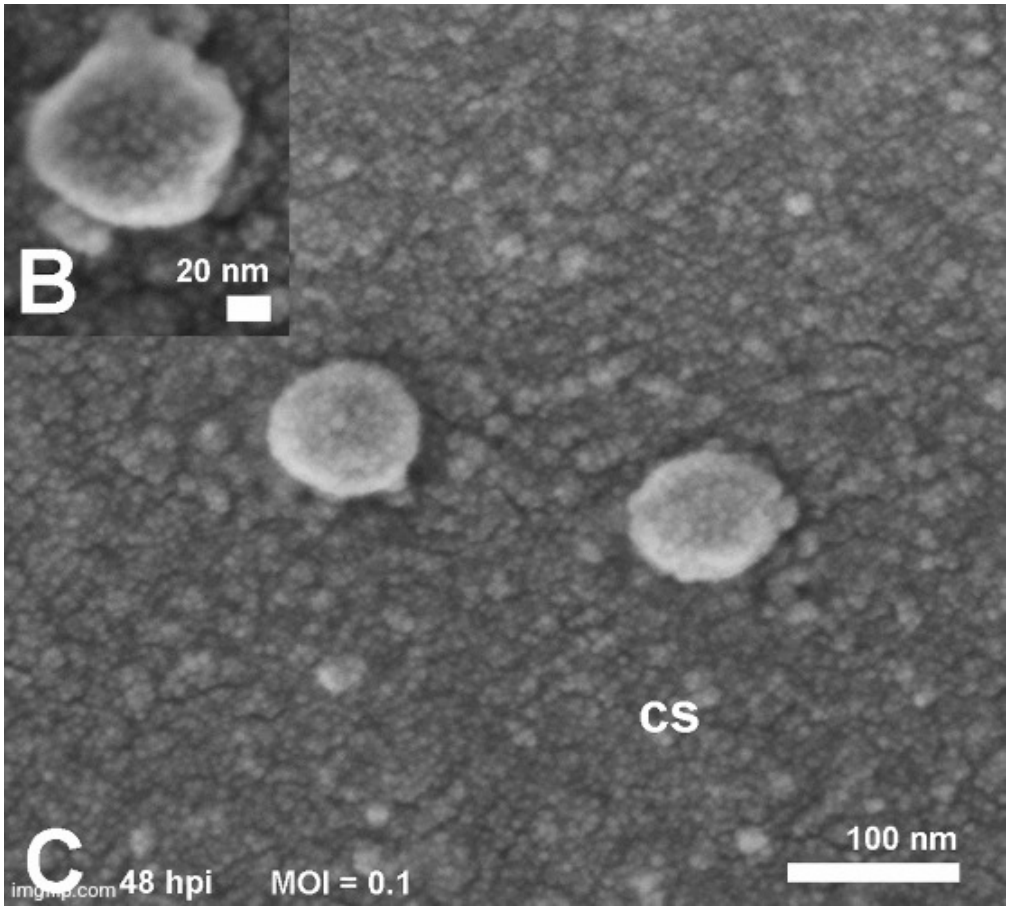


Fortunately for the WHO and the CDC, they have the convenient rescue device of "continued and rapid evolution" to explain away the contradictions and failures of the vaccines said to be based on this fictional piece of an imaginary "virus." They want you to believe that the vaccines will never be 100% effective as long as the spike protein can magically change itself inside the comput…er, body. Thus, newer and better vaccines created for the evolved spike protein along with regular boosters will be needed to combat the elusive nature of this highly intelligent protein endowed with shape-changing super powers. Any failures are due to the evolving particle, not the fraudulent approach.

Obviously, there are quite a few problems with this story beyond the inability to observe the fictional spike protein production process play out inside a living organism as well as the fantastical evolutionary powers of this tiny protein. If one were to think about this critically and logically, one would ask the same questions that should be asked about the evidence for the existence of any

“virus.” How was this spike protein discovered? Has this protein ever been observed in nature? Was it purified and isolated directly from the fluids of sick humans or was it a creation of the cell culture process? How was its functioning determined? Were proper controls carried out in any of the studies?

It should be clear to anyone who has ever looked into **the original papers supplied as evidence for the “coronaviruses” < https://viroliegy.com/2021/12/24/creating-the-coronavirus/>** that the spike protein is nothing but a creation based off of the staining patterns of random particles chosen as the representation for the alleged “virus.” Sometimes these spikes appear in the **electron microscope images < https://viroliegy.com/category/electron-microscope-images/>** and other times they do not (even though they are said to be there):

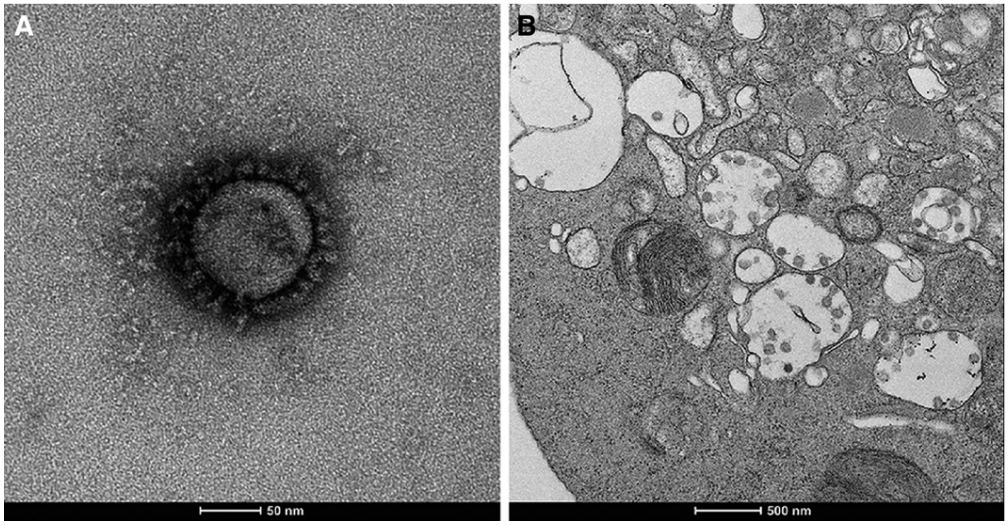


Discernible spikes?!?! **“Spikes** of SARS-CoV-2 particles observed on the cell surface **were discernible” https://www.nature.com/articles/s41598-020-73162-5/figures/2 < https://www.nature.com/articles/s41598-020-73162-5/figures/2>**

If the spikes are not observed, sometimes the sample is manipulated until they appear such as was done in this recent “SARS-COV-2” study from Australia where trypsin, a protein digestor, was added to alter the appearance of the imaged particles so that they contained the spikes:

> “Electron micrographs of the negatively stained supernatant showed spherical and pleomorphic virus–like particles of 90–110 nm diameter; the particles displayed prominent spikes (9–12 nm), characteristic of viruses from the family Coronaviridae (Box 5, A). Electron micrographs of sectioned VERO/hSLAM cells showed cytoplasmic membrane–bound vesicles containing coronavirus particles (Box 5, B) **Following several failures to**

recover virions with the characteristic fringe of surface spike proteins, it was found that adding trypsin to the cell culture medium immediately improved virion morphology."



There seem to be quite a few unstained spikeys floating off there by themselves…

https://www.mja.com.au/journal/2020/212/10/isolation-and-rapid-sharing-2019-novel-coronavirus-sars-cov-2-first-patient < https://www.mja.com.au/journal/2020/212/10/isolation-and-rapid-sharing-2019-novel-coronavirus-sars-cov-2-first-patient>

One thing that is for certain in the case of the creation of these spiked proteins is that the particles observed do not come from purified and isolated "viruses" found directly in the fluids of sick organisms. In fact, they are not even specific to "coronaviruses" at all as they can be observed on many extracellular vesicles such as clathrin-coated vesicles and **exosomes: < https://viroliegy.com/category/exosomes/>**

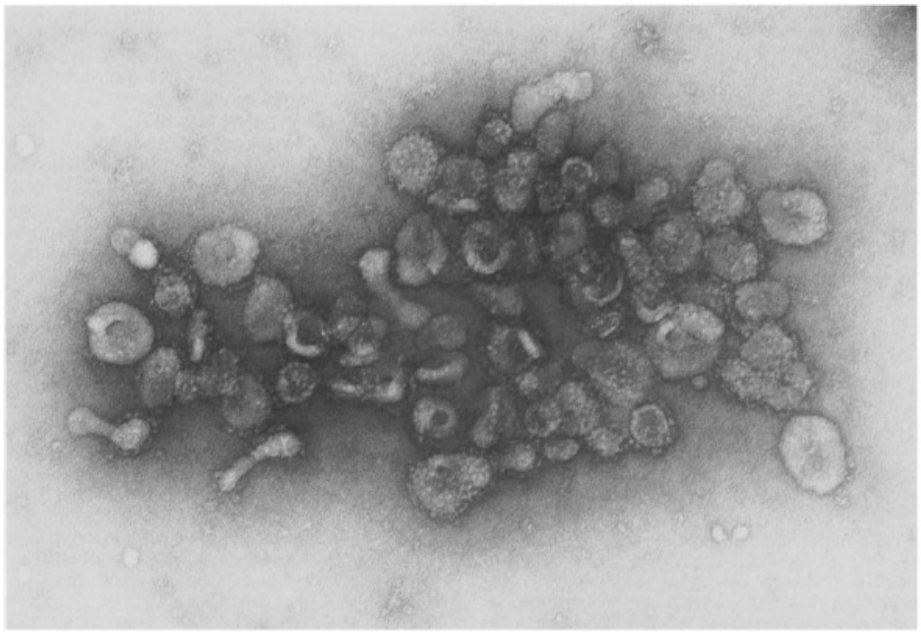
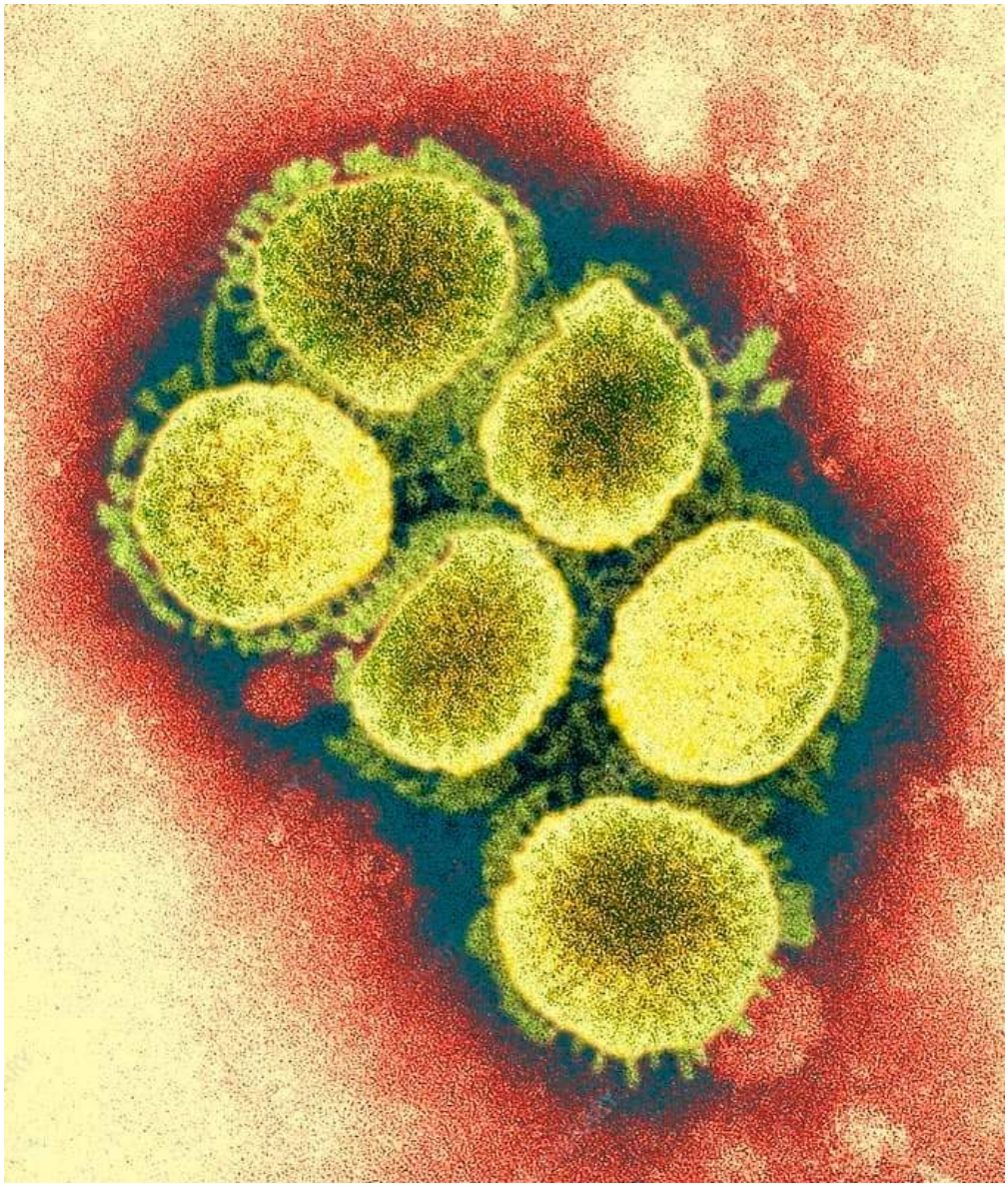


Figure 6. Vesicular Appearance of Released ^{125}I Material from ^{125}I-Antibody-Labeled Reticulocytes

After centrifugation at 12,000 × g to remove the cells, the supernatant was filtered through a Sepharose 6B column. The void volume was centrifuged at 100,000 g for 1 hr. The pellet obtained was stained with 1% ammonium molybdate and examined under an electron microscope. Vesicles with surface knobs are seen. Magnification: 123,000×.

Spikes on exosomes!!! doi: 10.1016/0092-8674(83)90040-5

Even the 2009 H1N1 swine flu gets some spiked love:



https://www.sciencephoto.com/media/83724/view/2009-h1n1-swine-flu-virus-tem# < https://www.sciencephoto.com/media/83724/view/2009-h1n1-swine-flu-virus-tem#>

The particles claimed to be spike proteins are a creation stemming from the **cell culture process < https://viroliegy.com/2021/09/05/the-case-against-cell-cultures/>** used to "isolate" the "viruses" as well as the preparations done in order to obtain the images by way of electron microscopy. As Harold Hillman said, the images obtained at the end of the process are far away removed from realty:

> "For example, most cytologists know, but readers of elementary textbooks do not, that when one looks at an illustration of an electron micrograph: an animal has been killed; it cools down; its tissue is excised; the tissue is fixed (killed); it is stained with a heavy metal salt; it is dehydrated with increasing concentrations of alcohol; it shrinks; the alcohol is extracted with a fat solvent, propylene oxide; the latter is replaced by an epoxy resin; it hardens in a few days; sections one tenth of a millimetre thick, or less, are cut; they are placed in the electron microscope, nearly all the air of which is pumped out; a beam of electrons at 10,000 volts to 3,000,000 volts is directed at it; some electrons strike a phosphorescent screen; **the electron microscopists select the field and the magnification which show the features they wish to demonstrate; the image may be enhanced; photographs are taken; some are selected as evidence. One can immediately see how far the tissue has travelled from life to an illustration in a book."**

https://www.big-lies.org/harold-hillman-biology/what-price-intellectual-honesty.htm < https://www.big-lies.org/harold-hillman-biology/what-price-intellectual-honesty.htm>

It should be clear that if none of the "coronaviruses" (ranging in size from 60 to 150 nm) have ever been properly purified, isolated, and separated from everything else in order to be independently studied, the much smaller surface spikes of the "virus" (ranging from 9 to 12 nm) have also never been properly purified and isolated in order to be independently studied. To confirm this, I did some digging into just the so-called **purification and isolation procedures < https://viroliegy.com/category/purification-isolation/>** relating to the spike itself. I came across a book by David Cavanagh, a man intimately tied to the study of this protein. He was involved in researching the "coronavirus infectious bronchitis virus (IBV)," focusing on the identification and molecular

characterisation of the “virion” proteins. In his book, he laid out the foundational papers for its supposed purification and isolation:

The Coronavirus Surface Glycoprotein

A. Electron Microscope Observations

> **“Coronaviruses are frequently claimed to have a characteristic morphology, including the possession of a “club-shaped” surface projection or spike (S) glycoprotein. However, in common with other aspects of the coronaviruses, the group exhibits variation with respect to the shape, size, and distribution of the S protein on the virion surface.** Davies and Macnaughton (1979) described the spikes of infectious bronchitis virus (IBV) and human coronavirus (HCV) 229E as being “tear-drop” shaped and widely spaced, whereas those of murine hepatitis virus (MHV) type 3 were mostly “cone-shaped” and closely spaced, although in some MHV-3 preparations the spikes were more bulbous. **Dimensions of S vary not only among the coronaviruses but also depending on the staining procedure;** following potassium phosphotungstate staining all three
> viruses had spikes approximately 20 nm long and 10 nm wide at the bulbous end, except for the cone-shaped spikes of MHV, which had a diameter of only 5 nm (Davies and Macnaughton, 1979). The entire S protein has been observed after solubilization and purification (Sturman et 01., 1980; Cavanagh, 1983c). The nonenvelope-associated SI subunit of the IBV S protein can become detached from the virion (Stern and Sefton, 1982a; Cavanagh and Davis, 1986).

B. Sedimentation Characteristics

> **Purification of the S protein of MHV, IBV, HCV strain 229E, bovine coronavirus (BCV), and porcine hemagglutinating encephalomyelitis virus (HEV) has been achieved using a combination of nonionic detergent and sucrose gradient centrifugation (Sturman et a1., 1980; Hasony and Macnaughton, 1981; Cavanagh, 1983b; Schultze et a1., 1990, 1991).** When milligram quantities of IBV were used, it was neeessary to dissociate and sediment the virus proteins in the presence of 1 M NaCl; otherwise the M protein cosedimented with S (Cavanagh, 1983b). **Spike has also been purified by affinity chromatography (Mockett, 1985; Daniel and Talbot, 1990).** Sedimentation studies have been variously interpreted as indicating that S from virions is a homodimer or homotrimer (IBV: Cavanagh, 1983eL homodimer (MHV: Vennema et a1., 1990b), or homotrimer (TGEV: Delmas and Laude, 1990)."

Click to access 10.1007%2F978-1-4899-1531-3_5.pdf < https://link.springer.com/content/pdf/10.1007%2F978-1-4899-1531-3_5.pdf>

It is not my intention to completely deconstruct each of the purification/isolation papers listed as it seems rather ridiculous to do so when the "viruses" these spike proteins supposedly come from have never been scientifically proven to exist. However, I do want to share some brief highlights from each paper so that it is abundantly clear the amount of manipulations and alterations that the sample must be put through in order to get the desired results. These "proteins" stem from the unpurified cell culture process where many substances are added and nothing is isolated. It will be clear to see how far removed from reality the sample has travelled in order to become an image published in a study. It stretches beyond belief to assume that the representative particles, imaged in a heavily altered dead and fixed state, have any bearing on actual

biological processes. They are nothing but a creation from the numerous processes utilized in order to obtain the final results.

1980

First up is the initial spike protein purification and isolation paper presented as evidence by Cavanagh. I highlighted the full methods section from this study in order to showcase the numerous steps involved to get to the final outcome. Many of the ensuing papers follow similar procedures in order to generate their outcomes so feel free to dig into each for more detailed accounts of the pseudoscientific processes employed.

What you will see in this paper is that the "virus" is a cell culture creation supplemented with the usual medium along with fetal bovine serum and antibiotics. The **fetal bovine serum < https://viroliegy.com/2021/08/31/the-unethical-use-of-fetal-bovine-serum/>** itself is a source of foreign genetic material along with other substances and compounds. The cell line used was the murine fibroblast cell line 17Cl-1, which, **according to BEI Resources, < https://www.beiresources.org/Catalog/cellBanks/NR-53719.aspx>** was "derived by spontaneous transformation of 3T3 cells. 3T3 is a nontumorigenic cell line established from 14- to 17-day-old embryos of the Balb/c mouse strain. 17Cl-1 cells are used for cultivation of murine coronaviruses, including murine hepatitis virus." Thus, we can see that this is already an unpurified concoction.

After culturing the "virus," it was "harvested" from the sample *before significant amounts of* ***cytopathic changes < https://viroliegy.com/2021/09/04/creating-the-cytopathic-effect/>*** such as cell fusion or lysis had occurred (the very criteria used to determine a "virus" is present) and then it was centrifuged. The sample was precipitated by the addition of 5.0 g of NaCl per 150 ml of clarified supernatant followed by a half volume of 30% polyethylene glycol. The "virus" was then put through a series of centrifugation steps with different chemicals and it was eventually radiolabeled with a uridine and amino acid mixture. NP40

was added to disrupt the "virions." According to **this manufacturer < https://www.sigmaaldrich.com/US/en/search/np-40?focus=products&page=1&perpage=30&sort=relevance&term=np-40&type=product_name>** , NP40 is a "non-ionic surfactant useful for the isolation and purification of functional membrane protein complexes. This detergent has been purified **to reduce levels** of contaminating aldehydes, metals, peroxides, and salts." In other words, more contaminants/foreign substances were added to "purify/idolate" the proteins.

Afterwards, the sample was subjected to the usual electron microscopy preparation procedures. The sample was also put through gel electrophoresis with a slew of additional treatment-mixture chemicals and then charged under high voltage for 4 hours in order to separate the proteins. Eventually, the researchers used theoretical antibodies created in similar unpurified manners to label and claim that the particles observed were the spiked ones that they were searching for. As is usually the case, no proper controls were outlined in this study:

Isolation of coronavirus envelope glycoproteins and interaction with the viral nucleocapsid

> "Studies of the virion polypeptides of a number of different coronaviruses in several laboratories have led to reports of three to a dozen or more polypeptide species associated with virions (1, 3, 7, 11, 16, 19, 23, 24, 28-31, 33,35, 44, 45, 48; 0. W. Schmidt and G. E. Kenny, Fed. Proc. 38:910, 1979). **The reasons for the apparent diversity in the polypeptide patterns of coronaviruses are not fully understood. Differences in technique and imperfect discrimination of virion polypeptides from those of host origin may account for some of the disparities. However, even when the same gel systems and conditions are employed in the same laboratory, significant differences in the number and size of virion polypeptides of**

different coronavirus strains have been observed (30). We propose that some of this apparent dissimilarity in the polypeptide patterns of coronaviruses may be the result of unusual characteristics of their envelope glycoproteins."

MATERIALS AND METHODS

"Cells and virus. A spontaneously transformed derivative of the BALB/c 3T3 cell line, designated 17 Cl 1, and the L2 derivation of the L929 cell line **were grown in Dulbecco medium supplemented with 10% unheated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 ,ug/ml).** The A59. strain of mouse hepatitis virus **was produced in 17 Cl 1 cells** and assayed by plaque titration in L2 cells as described previously (46).

Virus production and purification. 17 Cl 1 cell monolayers in glass roller bottles (120 by 260 mm; 690-cm2 cell surface area) were inoculated with A59 virus at a multiplicity of 1 to 10 PFU/cell. **After an adsorption period of 1 h at 37˚C, 50 ml of Eagle minimal essential medium with 10% unheated fetal bovine serum was added to each roller bottle. Cells were incubated at 37˚C.**

Released virus was usually harvested 24 to 26 h after inoculation, after several cycles of infection, when high yields were obtained **and well before significant amounts of cytopathic changes such as cell fusion or lysis had occurred.** Released virus was centrifuged at 10,000 x g (average) in a Sorvall GSA rotor for 30 min at 4˚C to remove debris. For optimal preservation of virus infectivity, freshly harvested virus was purified immediately at 4˚C without freezing or storage.

The virus was precipitated by the addition of 5.0 g of NaCl per 150 ml of clarified supernatant followed by a half volume of 30% polyethylene glycol to give a final concentration of 10% polyethylene glycol and 2.2% NaCl. The precipitate was collected by centrifugation at 10,000 x g (average) for 30 min at 4˚C, resuspended in TMEN 6 buffer (5 ml/3 roller bottles of original virus) containing 0.05 M Tris-maleate-0.001 M EDTA-0.1 ml NaCl (pH 6.0) at 4˚C, and layered over a discontinuous gradient of 4 ml of each of 30 and 50% (wt/wt) sucrose in TMEN 6 buffer in a 17.5-ml centrifuge tube or 3 ml of 30% and 2 ml of 50% (wt/wt) sucrose in TMEN 6 in a 12.5-ml centrifuge tube. After centrifugation for 4 h at 25,000 rpm in a Spinco SW 27.1 rotor (82,000 x g, average) or 3 h at 30,000 rpm in a Spinco SW 41 rotor (110,000 x g, average) at 4˚C the narrow white virus band at the interface between 30 and 50% sucrose was collected, diluted 2.5-fold with TMEN 6 buffer, and layered over a 7.5- or 12-ml continuous gradient of 20 to 50% sucrose in TMEN 6 buffer. This gradient was centrifuged for 18 h at 25,000 rpm (82,000 x g, average) at 4˚C on a Spinco SW 27.1 rotor or 30,000 rpm in a Spinco SW 41 rotor (110,000 x g, average), and the virus band at 1.17 to 1.19 g/ml was collected. The virus was then diluted with TMEN 6 buffer and either used directly, pelleted in an SW 50.1 Spinco rotor, or dialyzed against buffer or water. Data from a representative virus purification (Table 2) shows a 50- to 100-fold reduction in volume and greater than 100-fold decrease in total protein, with 60 to 100% recovery of virus infectivity. The final specific infectivity achieved was 5 x 10’0 to 10 x 1010 PFU/ml of protein.

As a routine practice, up to 900 ml of virus was purified at one time. This is the volume obtained from 18 roller bottles of infected cells. Virus harvested at 24 h had a titer of 2 x 10^8 to 5 x 10^8 PFU/rnl, and at 42 to 48 h the titer

was 0.5 x 10^9 to 2 x 10^9 PFU/ml. Thus, depending on the time of harvest, yields of released virus were 10^10 to 10^11 PFU/roller bottle.

Radiolabeling of virus. Radiolabeled compounds **were added to the medium in final concentration of 2 to 4 uCi of L-3H-amino acid mixture, or [5-3H]uridine per ml, 3 uCi of [6-3H]fucose per ml, and 1 to 4 uCi of [35S]methionine per ml.** Infected cultures were incubated in the presence of these compounds from approximately 1 h after virus inoculation until virus was harvested. **Labeled uridine and amino acid mixture** were purchased from New England Nuclear Corp., Boston; labeled methionine and fucose were obtained from Amersham Corp., Arlington Heights, Ill **[3H]-uridine-labeled 28S HeLa cell rRNA** was kindly provided by N. K. Chatterjee.

Disruption of virions with NP40. Purified virions from three or fewer roller bottles were usually pelleted and resuspended in 1 to 2 ml of TMEN 6 buffer at 4˚C. **NP40, kindly provided by Shell, Inc., was added to a final concentration of 0.25 to 1%, and the mixture was shaken vigorously by hand at least 20 times.** In some experiments a portion of the detergent-treated virus was incubated at 37˚C for 30 min at this stage. **The detergent-treated virions were then layered at 4˚C over 15 to 50% sucrose gradients in TMEN 6 buffer containing 0.1% NP40 or at 10˚C over 30 to 75% sucrose gradients in TMEN 6 buffer containing 0.1% NP40.** A cushion of 76% Renografin or 65 to 75% sucrose was placed beneath the 15 to 50% sucrose gradient. Unless otherwise indicated, the gradients were sedimented at 38,000 rpm (180,000 x g, average) for 16 to 20 h in an SW 41 rotor at 4 or 10˚C. Gradient fractions were collected from the top of the gradient with an ISCO fractionating device or from the bottom by displacement with light

paraffin oil delivered with a Cornwall syringe. Gradient fractions were collected into chilled tubes and held at 4˚C. Samples for radioisotopic counting were prepared by transferring 50- to 450-pd portions of each gradient fraction into 1 ml of water-10 ml of Aquasol (New England Nuclear). The refractive indexes of gradient fractions were determined with a Bausch and Lomb Abbe refractometer. **Continuous 20 to 50% gradients of Renografin-76 (E. R. Squibb & Sons, Princeton, N.J.) containing 0.1% NP40 were employed for dissociation of nucleocapsid complexes.** Sedimentation was carried out at 25,000 rpm (82,000 x g, average) for 16 h in an SW 27.1 rotor at 4˚C.

Electron microscopy. Samples of purified glycoprotein preparations were placed on carbon-coated Formvar-covered 400-mesh copper grids, negatively stained with 2% phosphotungstic acid at pH 7.2 and examined with a Philips 400 transmission electron microscope.

SDS-polyacrylamide gel electrophoresis. The method of high-pH discontinuous buffer SDS-polyacrylamide gel electrophoresis in cylindrical gels employed in this study has been described previously (44). Because the El polypeptide aggregates after boiling with SDS in the presence of mercaptoethanol (44), samples of viral polypeptides for this study were prepared for electrophoresis by heating at 37˚C for 30 min in the absence of mercaptoethanol. Five to twenty percent polyacrylamide gradient slab gels 1.5 mm thick and 10 cm long were prepared by the method of Laemmli in a Hoeffer slab gel electrophoresis apparatus. **Each well was loaded with approximately 40 ul of a radiolabeled sample which had been heated to 37˚C for 15 min with an equal volume of sample treatment mixture composed of 6 M urea, 4% SDS, 0.05% bromophenol blue in 0.0625 M**

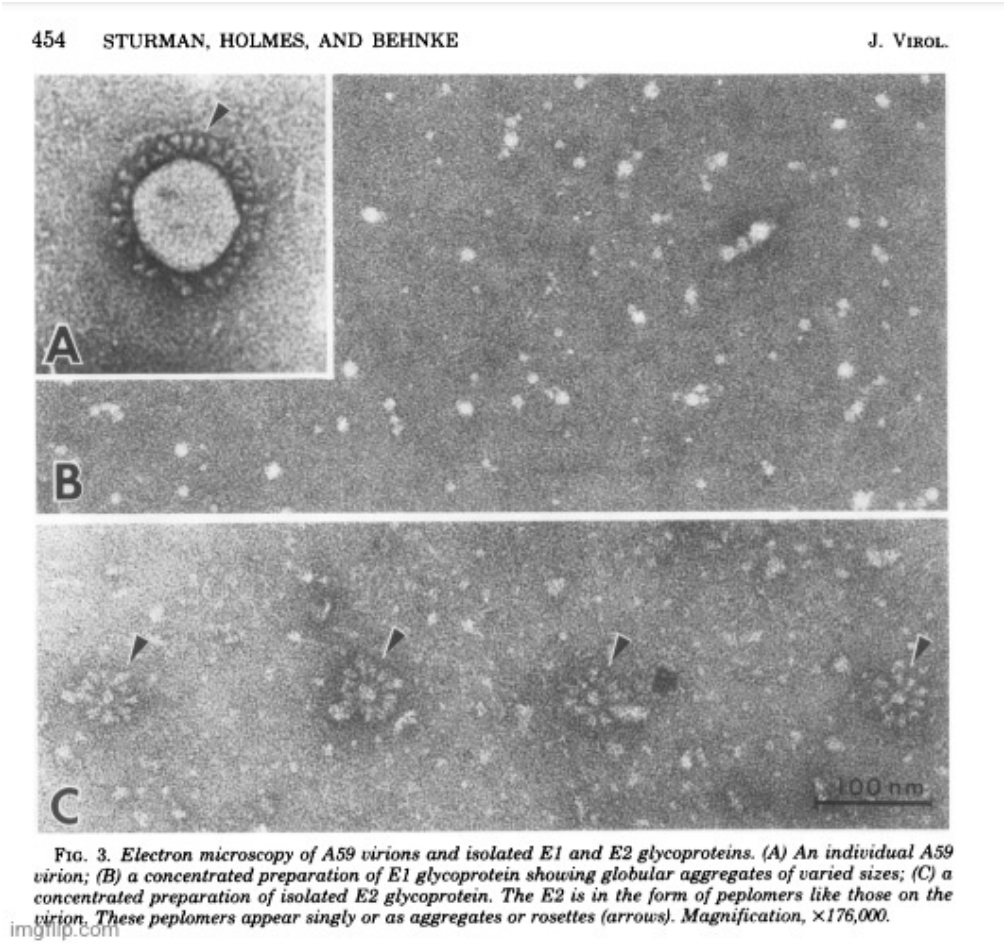
Tris-chloride, pH 6.7. Gels were run at 125 V for about 4 h under constant voltage from a Savant power supply. Gels were impregnated with PPO (2,5-diphenyloxazole; Sigma) in dimethyl sulfoxide by the method of Bonner and Laskey (4), dried with a Savant gel drier onto Whatman no. 17 chromatography paper, exposed to Kodak XR-5 film at -70°C for 1 to 4 weeks, and developed with Kodak X-ray chemicals.

Preparation of antisera. Antiserum directed against A59 virion polypeptides were prepared by purifying released virus through discontinuous and continuous sucrose gradients as described above without prior precipitation with PEG. **Purified, concentrated A59 virus was disrupted with 1% NP40 and frozen at -75°C in aliquots. A 0.1-ml amount of the virus preparation was inoculated with complete Freund adjuvant into rabbit footpads, followed after 2 weeks with a second footpad injection of an additional 0.1 ml with incomplete Freund adjuvant (Difco) and 1 week later by an intravenous injection of another 0.4 ml of detergent-treated virus. After the third injection, rabbits were bled from the ear at 1-week intervals for 1 month.** This antibody specifically immunoprecipitated radiolabeled El, N, and E2 and no cellular polypeptides from NP40 extracts of infected 17 Cl 1 cells.

Antisera against the isolated El and E2 proteins were prepared by a similar schedule of rabbit inoculations of El obtained from sucrose gradient sedimentation of A59 virions disrupted with NP40 at 4°C and of E2 from NP40-disrupted virus that had been incubated at 37°C before sucrose gradient sedimentation. These antisera were each passed through immunosorbent columns of Affigel 10 charged with the other viral glycoprotein. The specificity of the antisera was determined by

immunoprecipitation of radiolabeled viral polypeptides from NP40 extracts of A59-infected cells.

Immunoprecipitation of viral polypeptides. Immunoprecipitates of radiolabeled viral polypeptides
from extracts of infected cells, purified virions, or gradient-purified El or E2 were made by the method of Kessler (26). **A 25-ul amount of rabbit serum was incubated with 25 to 200 ul of radiolabeled sample in phosphate-buffered saline containing 0.1% NP40 at 0°C for 1 h. Antigen-antibody complexes and antibody were precipitated with an excess of purified, Formalin-fixed staphylococci (Cowan 1 strain) for 10 min at 4°C, pelleted at 3,000 rpm for 10 min, washed three times in 1 ml of 0.05% NP40 with phosphate-buffered saline and then solubilized in sample treatment mixture by boiling for 1 min or treatment at 37°C for 15-30 min."**



The spike proteins in the bottom image created the exact same circle and spike pattern as in the original “viral” particle. That couldn’t be a result from the procedures now could it…? 🤔

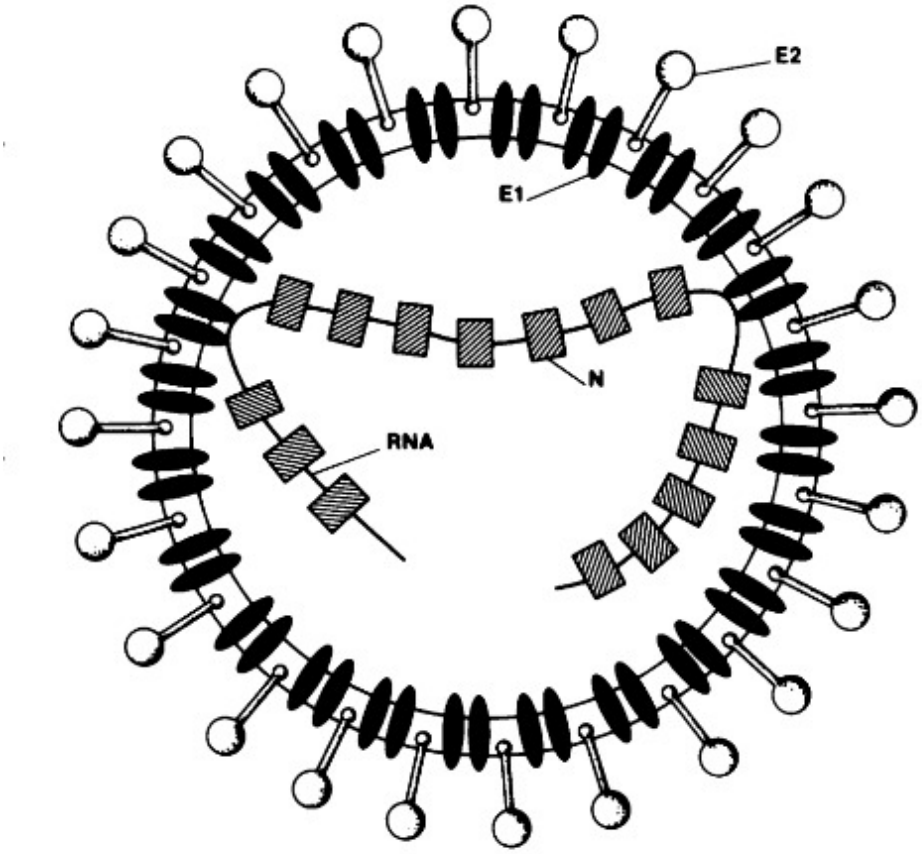


FIG. 11. *Schematic model of the location of E2, E1, and N proteins in relation to the viral envelope and RNA.*

"The experimental evidence obtained here and in previous studies **suggests a model** for the arrangement of components in the coronavirus A59 virion (Fig. 11)."

doi: 10.1128/JVI.33.1.449-462.1980

1981

This next study followed similar procedures as the first. I've highlighted the "virus" growth and "purification" methods in order to show that this purification/isolation claim does not hold up under scrutiny. The "virus" was grown in mouse embryonic fibroblasts and incubated in Eagle's minimal essential medium with fetal calf serum. The "virus" was centrifuged and "purified" by methods "previously discussed." The rest of the techniques used are similar to those listed in the 1981 study and the results are heavily reliant on

serological data where it was apparently difficult to ensure similar **antibody responses < https://viroliegy.com/category/antibodies/>** in the mice tested as there was considerable variation observed. No EM images of the "purified/isolated" spike proteins accompany the study:

Antigenieity of Mouse Hepatitis Virus Strain 3 Subeomponents in C57 Strain Mice

Materials and Methods

Virus Growth

> **"MHV3 was grown in confluent secondary mouse embryonic fibroblasts.** Monolayers were infected at an input multiplicity of 0.1 infectious particles per cell and following an adsorption period of 1.5 hours at 37 ˚ C, **were incubated for 72 hours at 37 ˚ C in Eagle's MEM with 2 percent foetal calf serum (13).** Aliquots of this virus suspension were stored at –70˚C and **used for the preparation of purified virus particles and subcomponents.**

Preparation of Purified Virus

> Virus was purified at 0 ˚ to 4 ˚ C as described previously (13). **The virus was pelleted at 75,000 × g for 1 hour and then resuspended in 1 ml Dulbecco's phosphate buffered saline "A" (PBSA).** The resuspended virus was overlaid on to a linear 25 to 55 percent (w/w) sucrose gradient in PBSA and centrifuged for 16 hours at 90,000 × g. The virus peak at 1.18 g/ml was collected."

Diseussion

> "In this paper we report the isolation and purification of MHV 3 subviral components and have shown the role of each subcomponent in the protection of immnnised mice against challenge with infectious MHV 3. **It was difficult to ensure that mice immunised with different virus subcomponent preparations all produced comparable amounts of antibody, as there was considerable variation in the immunogenieity of the subeomponents. The highest antibody rises detected by ELISA were directed against surface projections, while lower antibody rises were observed against membrane and RNP, suggesting that the most immunogenic part of the virus is an antigen(s) associated with the surface projections.** Similar results have been obtained previously with human eoronaviruses (14) and the porcine coronavirus transmissible gastroenteritis virus (TGEV) (5)."

doi: 10.1007/BF01315263.

1983

In this study, the "virus" was grown in the chorioallantoic membrane (CAM) cells of de-embryonated chicken eggs and radiolabelled IBV-M41 was prepared in pairs of de-embryonated eggs and treated. The same ultracentrifugation of impure cultured material was utilized to claim purity and isolation even though it is well known that this technique can not separate particles of the same size, shape, and density from one another. It is admitted in the study that there is less agreement among researchers on the composition of the S protein. It was also admitted that other polypeptides of 110K and 75K were detected in "virus" preparations which they assumed were probably host polypeptides. No EM images of the purified and isolated particles accompanied the study nor were proper controls carried out:

Coronavirus IBV: Further Evidence that the Surface Projections are Associated with Two Glycopolypeptides

"The avian infectious bronchitis virus (IBV) particle, like other coronaviruses, contains three
major protein structures, the surface projection or peplomer (S), nucleocapsid (N) and matrix (M) proteins (Cavanagh, 1981 ; Siddell et al., 1982). The M protein comprises a polypeptide of mol. wt. 23 000 (23K) which is glycosylated to different extents to form glycopolypeptides of mol. wt. up to 36K (Stern et al., 1982; Stern & Sefton, 1982; Cavanagh, 1983). A polypeptide of 50K to 54K forms the N protein (Macnaughton et al., 1977). **There is less agreement on the composition of the S protein. Since the presumptive S polypeptides of all coronaviruses examined have mol. wt. greater than that of the N polypeptide,** the following account refers only to such IBV polypeptides."

"Radiolabelled IBV-M41 was prepared in pairs of de-embryonated eggs (Cavanagh, 1981); each egg received 125 uCi [35S]methionine (sp. act. > 800 Ci/mmol) or [35S]methionine plus 165 uCi of a mixture of 15 3H-labelled amino acids (code TRK 440, Amersham International). Unlabelled virus was grown in batches of 200 11-day-old embryonated eggs which were inoculated with approx. 3-5 log10 median ciliostatic dose50 of IBV-M41. After 24 h at 37 °C the eggs were chilled at 4 °C overnight. Allantoic fluid was collected, clarified at 4000 g for 30 min and the virus pelleted at 35000 g for 2-5 h. **The pellet was resuspended in NET buffer (100 mM- NaC1, 1 mM-EDTA, 10 mM-Tris-HCl pH 7.4) to a vol. of 20 ml and sonicated at maximum amplitude for 10 s with the 3 mm probe of an MSE ultrasonic disintegrator.** The suspension was placed on two discontinuous gradients comprising 20 m125% (w/w) sucrose and 5 m160%

(w/w) sucrose in NET in a 6 x 38 MSE swing-out rotor. After centrifugation at 65,000 gmax for 2.5 h at 4 ˚C, the gradients were fractionated. Fractions which contained virus at the 25/60% sucrose interphase **were pooled, diluted threefold, placed on a 25 to 55 % (w/w) sucrose gradient in NET** and centrifuged at 50000 gay for 16 h at 4 ˚C in a 6 x 38 rotor. Fractions of 1 ml were collected and those of density 1.16 to 1.22 g/ml **were pooled, diluted to 20% (w/w) sucrose** and the virus pelleted at 90000g for 3 h at 4 ˚C in an MSE 3 x 25 swing-out rotor. Pelleted virus (2 to 4 mg protein) was **resuspended in 1 ml l M-KCl or 1 M-NaC1 in NET and 1 ml 4% (v/v) Nonidet P40 (NP40) in NET containing 1 M-KCl or 1 M-NaCl, followed by sonication for 2 to 3 s and incubation at 25 ˚C for 1 h.** Undissolved material was removed by low-speed centrifugation and the supernatant **placed on a 10 to 55% (w/w) sucrose gradient in NET containing 1 M-KC1 or 1 M-NaC1 and 0.1 ~ NP40.** After centrifugation in an MSE 6 x 38 swing-out rotor at 85,000 gav for 16 h at 4 ˚C fractions of 500 pi were collected. These were dialysed to remove KCl where appropriate prior to electrophoresis. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in tubes and slabs with a 10% acrylamide resolving gel (Cavanagh, 1981). Samples were dissociated at room temperature with 2% SDS and 2% (v/v) 2-mercaptoethanol. **Unlabelled markers used were phosphorylase b, bovine serum albumin and carbonic anhydrase; some phosphorylase was pre-stained with Drimarine brilliant blue K-BL (Bosshard & Datyner, 1977) and the apparent mol. wt. was 110K."**

"Thus, these studies show that the peplomers of IBV comprise two glycopolypeptides of 90K and 84K in equimolar proportion. **The polypeptides of 110K and 75K, variably detected in virus preparations, are probably host polypeptides."**

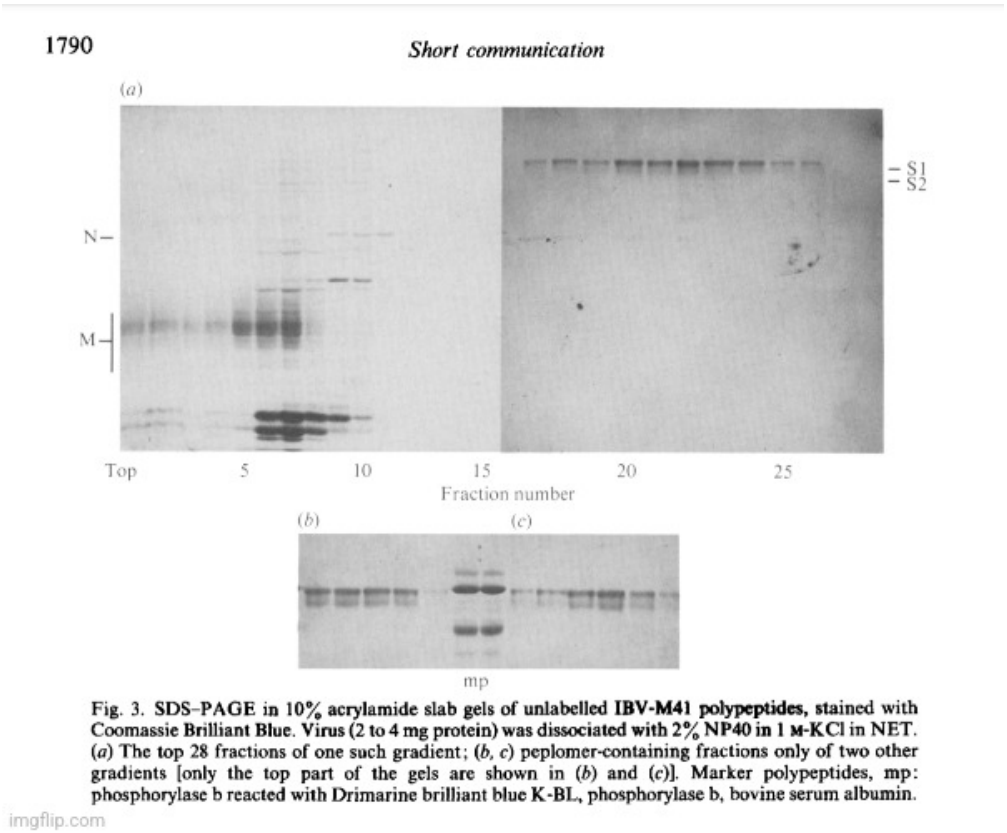


Fig. 3. SDS–PAGE in 10% acrylamide slab gels of unlabelled IBV-M41 polypeptides, stained with Coomassie Brilliant Blue. Virus (2 to 4 mg protein) was dissociated with 2% NP40 in 1 M-KCl in NET. (*a*) The top 28 fractions of one such gradient; (*b*, *c*) peplomer-containing fractions only of two other gradients [only the top part of the gels are shown in (*b*) and (*c*)]. Marker polypeptides, mp: phosphorylase b reacted with Drimarine brilliant blue K-BL, phosphorylase b, bovine serum albumin.

doi: 10.1099/0022-1317-64-8-1787.

1985

In this study, affinity chromatography was used to “purify” cell cultured “viruses.” However, keep in mind that it is known that this technique can not separate “viruses” from exosomes or other particles with the same size, shape, and density. The researchers also rely on theoretical monoclonal antibody reactions to identify the “viral” proteins from within a crude mixture of other proteins which are said not to be “viral” proteins based on the **non-specific measurement of the theoretical antibodies < https://viroliegy.com/2021/11/12/antibody-specificity/>** . It is mentioned that the there were other stained bands present during gel electrophoresis and that these are artifacts sometimes observed, even in the absence of protein, with this staining procedure. No proper controls were outlined and the only image was of the bands from the gel electrophoresis experiments:

Envelope proteins of avian infectious bronchitis virus: Purification and biological properties

Virus

The Massachusetts M41 strain of IBV was **grown in the allantoic cavities of 11-day-old embryonated chicken eggs** and purified on isopycnic sucrose gradients as described by Cavanagh (1981).

Preparation of material for affinity chromatography

Purified virus was pelleted in a 6 X 14 ml rotor at 70,000 X g for 3 h at 4°C and resuspended phosphate-buffered saline (PBS). **An equal volume of PBS containing 4% (wt./vol.) NP40 was added, mixed using a Dounce homogeniser and incubated for 2 h at 25°C.** The material was centrifuged for 5 min in an Eppendorf microcentrifuge and the resulting supernatant, containing soluble viral components, was used for the affinity chromatography purification.

Immunoadsorbent preparation

Monoclonal antibodies (designated A38 and C24) to the spike and membrane proteins respectively of IBV strain M41 were prepared (Mockett et al., 1984). The
gammaglobulin fraction of ascitic fluids containing either anti-spike or anti-membrane monoclonal antibodies was isolated by salt precipitation using a final concentration of 18% (wt./vol.) Na_2SO_4. For the spike immunoadsorbent 5.6 mg of gammaglobulin was coupled to 0.75 mg of CNBr-Sepharose 4B (Pharmacia) according to the manufacturers' instructions and for the membranc immunoadsorbent 5.5 mg was coupled to the same amount of gel. **Unreactive groups on the gel were blocked**

using 1 M ethanolamine, pit 8.0, and any non-covalently bound proteins were removed by repeated washings with 0.1 M NaHCO~ buffer, pH 8.3, containing0.5 M NaCl and 0.1 M acetic acid buffer, pH 4.4, containing 0.5 M NaCl. The immunoadsorbent was stored in PBS containing 0.2% NaN3 at 4˚C until used. It was washed twice with 3 M NH4SCN in PBS containing 0.1% octylglucoside, four times with PBS and twice with PBS containing 2% NP40 before use. All wash volumes were 10 ml.

Affinity chromatography

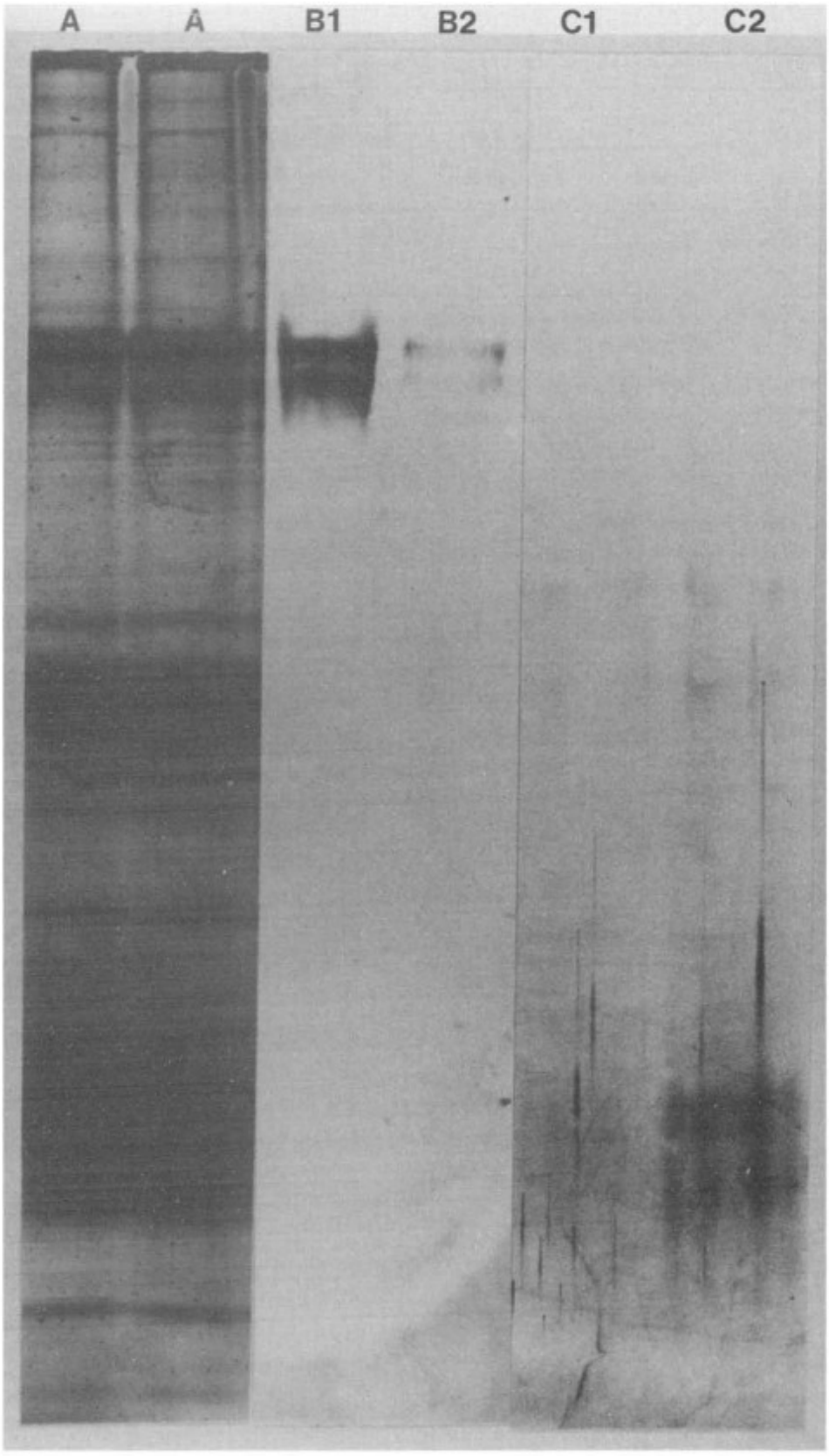
The solubilised virus preparation **was mixed with the immunoadsorbent** for 16 h at 4˚C using a rotary stirrer. **The gel was poured into a chromatography column and washed with PBS containing 0.1% NP40 (40 ml) and PBS containing 0.1% octylglucoside (10 ml). 3 M NH,SCN in PBS containing 0.1% octylglucoside was added and 10 fractions of 1 ml collected.** The absorbance at 280 nm of each of the fractions was read using a SP1800 PyeUnicam spectrophotometer. The fractions in the absorbance peak were dialysed against PBS. A sample of each fraction was then subjected to electrophoresis in a polyacrylamide gel. **Those fractions containing detectable viral protein were pooled and constituted the purified protein preparation."**

"The viral proteins purified by affinity chromatography are shown in Fig. 1. The spike protein, which is composed of two polypeptides, was the only protein detected in the two fractions shown using the sensitive silver staining procedure. Similarly the membrane protein was not contaminated with other proteins, although this protein did not stain as well as the spike. **There were other stained bands present, but these are artifacts**

sometimes observed, even in the absence of protein, with this staining procedure."

This paper describes the application of affinity chromatography **using monoclonal antibodies for the purification of the two viral structural proteins present at the surface of the IB virion** – spike and membrane. A previous report has described procedures for the purification of these viral proteins and also nucleocapsid protein, the only other major structural protein (Cavanagh, 1983). **IBV was solubilised in NP40 detergent and centrifuged in a sucrose gradient containing this detergent in order to purify the nucleocapsid protein. The addition of 1 M NaCl to the sucrose solutions was required for the purification of the spike and membrane proteins, as they co-migrated in gradients containing low salt concentrations. However, the nucleocapsid protein could not be purified in gradients containing high salt concentrations.** The yield of material from these gradients was relatively low, due to the limited number of fractions which contained purified viral components. **In other studies (Cavanagh, 1984) purified spike material contained some nucleocapsid protein and the membrane preparation contained other proteins which were thought to be of cellular origin.**

There are a number of advantages in using affinity chromatography. **By making use of the specificity of the antibody pure material can be isolated, even from a crude mixture of proteins.** The method is very quick and easy and the immunoadsorbent can be used several times. Thus, relatively large amounts of purificd material can be obtained. The availability of spike and membrane proteins in a highly purified form will allow more biochemical, structural and immunological studies to be done."



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1990

In this first of two studies from 1990, affinity chromatography is once again used to attempt "purification" from cell culture supernatant. This time the cells used were in the form of DBT which are mouse brain tumor cells said to be transformed by Rous Sarcoma "virus." The MHV "virus" was passaged four times and was produced in medium containing fetal calf serum which, again, is a source of many host/cellular components itself. Various other chemical additives were used throughout the processes and in the end, it was admitted that "non-viral" host material was reproducibly present in the samples. No EM images of the purified/isolated spike proteins were shown beyond gel electrophoresis stains:

Protection of Mice from Lethal Coronavirus MHV-A59 Infection by Monoclonal Affinity-Purified Spike Glycoprotein

> **"Cells and virus**
> The A59 strain of MHV (MHV-A59), **obtained from the American Type Culture Collection (**Rockville, MD, U.S.A.), was plaque-purified twice **and passaged four times at a multiplicity of infection (MOl) ofO.Ol on DBT cells.**
>
> **Antigen preparation**
> Virus was produced as described previously **in culture medium containing 1 % (v/v) FCS.** DBT cell monolayers were infected with MHV-AS9 at an MOl of 0.01 and medium was harvested 16 hrs post-infection. **Cell debris were pelleted and virus concentrated by precipitation with 10% (w/v) polyethyleneglycol in O.S M NaCl. Viral antigens were resuspended and**

dialyzed against TMEN buffer (0.1 M Tris-acid-maleate, pH 6.2, 0.1 M NaC!, 1 mM EDTA), and kept at -70˚C until used. In some experiments, virus was labeled by adding 4 mCi of [3SS]methionine (ICN Biochemicals Canada, Ville St-Laurent, PQ, Canada) to culture medium at 6 hrs post-infection.

Affinity chromatography

The E2/S-immunoadsorbent was prepared by coupling five milligrams of purified MAb 7-10A 11 to 1 g of CNBr-activated Sepharose 4B (Pharmacia, Dorval, PQ, Canada), all steps performed according to manufacturer's instructions. **For affinity chromatography, concentrated virus was solubilized with 2% (v/v) Nonidet P-40 (NP-40) for 2 hrs at room temperature (RT), and soluble proteins were mixed with the 7-lOA-Sepharose gel and incubated end-over-end for 16 hrs at 4˚C. The gel specificity was determined by immunoadsorption of radiolabeled antigen, extensive washing with 0.1 % (v/v) NP-40 (in 0.2 M phosphate buffer, pH 6.2, 0.1 M NaC!, 1mM EDTA), and elution of adsorbed proteins into electrophoresis sample buffer.** Otherwise, the gel was poured into a column and washed until the absorbance at 280 nm had dropped to baseline level, after which the column was washed with 4 gel volumes of the same buffer containing 0.1 % (w/v) of octylglucoside for detergent replacement. **Elution was carried out by adding 3 M ammonium isothiocyanate to the latter solution. Fractions of 1 ml were collected and dialyzed against O.O5 M ammonium bicarbonate, pH 7.4.** A sample of each fraction was lyophilized, resuspended in electrophoresis sample buffer, and analyzed on a 7-1S% linear polyacrylamide gel, prior to fluorography with Enlightning® (Dupont Canada, Lachine, PQ, Canada) for radiolabeled antigen or silver stainingl2. **Fractions containing purified E2/S were pooled and used for immunological studies."**

Purification and immunogenicity of E2/S glycoprotein

The E2/S glycoprotein used for immunogenicity studies was purified **from viral antigens concentrated from 1.8 liters of culture medium from MHV -A59-infected DBT cells.** Fractions eluted after immunoaffinity chromatography were analyzed by SDS-PAOE and silver staining. **Figure 2 shows that the dimeric and monomeric forms of E2/S were purified without detectable contamination from other viral proteins. However, a contaminant (30 kDa), which was probably of cellular origin, was reproducibly observed.** The purified glycoprotein was partially denaturated, as confirmed by its loss of reactivity with the MAb 7-10A (data not shown)."

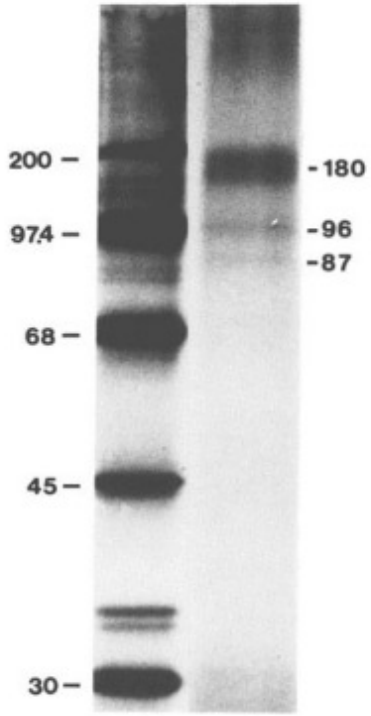


Fig. 1. Immunoadsorption of [^{35}S]methionine-labeled antigen on 7-10A-Sepharose gel. Lane M, molecular mass markers (kDa); Lane 1, proteins eluted from the affinity gel.

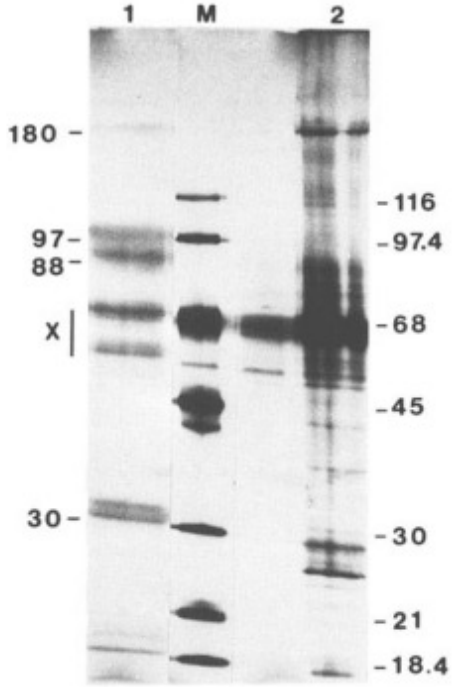


Fig. 2. Silver-stained SDS-PAGE of immunoaffinity-purified E2 glycoprotein. Lane M, molecular mass markers (kDa); Lane 1, proteins eluted from the affinity gel; Lane 2, antigen applied on gel. The unnumbered lane contains only PAGE sample buffer and shows some artefactual bands

(X) reproducibly observed in our silver-stained reducing SDS-PAGE.

https://link.springer.com/chapter/10.1007/978-1-4684-5823-7_28 < https://link.springer.com/chapter/10.1007/978-1-4684-5823-7_28>

This second study from 1990 is mostly a serolgical study which also used cell culturing techniques to aquire the "virus" being studied. HEV was said to be "isolated" by nasal swab from a pig where it was grown in MDCK I cells from a dog and "purified" by centrifugation through a sucrose gradient. Keep in mind that sucrose gradient centrifugation, even though it is considered the "gold standard," can not completely purify/isolate particles of the same size, shape, and density. There were no EM images of the spike proteins beyond the gel electrophoresis ink blots presented below. The proteins were claimed to be detected serologically based on stains. The methods used to obtain the results were also poorly defined with no apparent controls:

Isolation and characterization of the acetylesterase of hemagglutinating encephalomyelitis virus (HEV).

> "HEV is related to BCV both serologically and in its hemagglutinating properties. We analyzed whether HEV also has acetylesterase activity like BCV strain NT-9 of HEV, **which has been isolated by nasal swab from a pig (Heb and Bachmann, 1978), was grown in MDCK I cells and purified by centrifugation through a sucrose gradient.** The purified virus was analyzed for its ability to release acetate from p-nitrophenylacetate (PNPA)."

> **"Purified HEV was incubated with 3H- DFP for 30 min at 4°C and then analyzed by SDS-polyacrylamide gel electrophoresis.** The result is shown in Fig. 2. **Following staining with Coomassie Brilliant Blue the viral**

proteins N, M, S, and HE became visible. The radioactive label was found to comigrate only with the latter glycoprotein. The identity of the 3H-DFP-labeled protein was confirmed by analyzing the sample in the absence and presence of reducing agents. Under non-reducing conditions the labeled protein was detected in a position expected for a protein with a molecular weight of about 140 kDal. In the presence of dithiotreitol the apparent size is reduced to about 65 kDal indicating that in the native protein monomers are connected by disulfide bonds to form a dimeric structure. This migration behavior is characteristic for the viral protein involved in the hemagglutinating activity of HEV (Callebaut and Pensaert, 1980) and therefore, in analogy to BCV, is designated HE.

In order to isolate the esterase of HEV from the viral membrane, purified virions were treated with 1% octylglucoside (OG). The nucleocapsid as well as the M-protein were pelleted by centrifugation for 30 min at 25.000 x g (not shown). The glycoproteins remaining in the supernatant (S and HE) were loaded onto a 10-30% sucrose gradient in PBS containing 1% OG. Following centrifugation at 42.000 rpm for 16 h in SW55 rotor, fractions were collected from the bottom of the tube and analyzed by SDS-polyacrylamide gel electrophoresis. As shown in Fig. 3, S-protein was detected in fraction 3, while most of HE was recovered from fraction 6. Analysis of the fractions for acetylesterase activity revealed that only fractions containing HE were able to release acetate from PNPA. This result confirms that HE is responsible for the esterase activity of HEV."

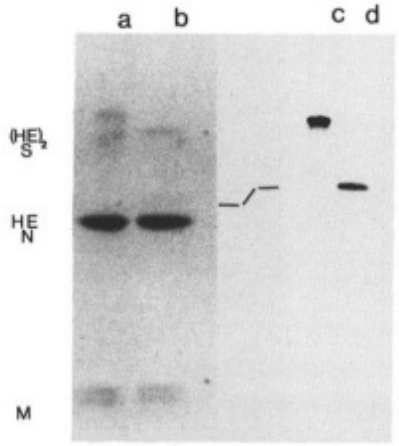


Fig. 2. Identification of the esterase protein of HEV. Purified virus was incubated in the presence of ^{3}H-DFP for 30 min on ice and then analyzed by SDS-polyacrylamide gel electrophoresis in the absence (lanes a and c) and presence (lanes b and d) of dithiotreitol. The gel was stained with Coomassie Brilliant Blue (lanes a and b) and processed for fluorography (lanes c and d)

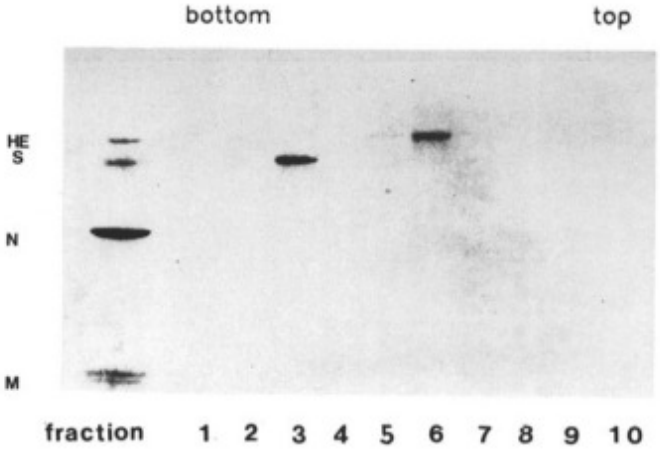


Fig. 3. Purification of the acetylesterase of HEV. The supernatant of octylglucoside-treated virions was centrifuged through a sucrose gradient (see text) and the fractions were analyzed by SDS-polyacrylamide gel electrophoresis under non-reducing conditions.

https://link.springer.com/chapter/10.1007/978-1-4684-5823-7_16 <
https://link.springer.com/chapter/10.1007/978-1-4684-5823-7_16>

1991

In this last study, BCV was grown in MDCK I cells, a subline of Madin-Darby canine kidney cells. Once again, ultracentrifugation was used to "purify" the already contaminated and impure concoction with the addition of phosphate buffered saline and other chemicals such as n-octylglucopyranoside, a nonionic detergent used for membrane protein solubilization. The researchers then used EM images to determine that the S protein, designated as the long projection arm of the "virus," must be reasonably assumed to be used by the "virus" to attach to cells in order to hijack them as it will encounter the cell first… *as it is longer.*

The S Protein of Bovine Coronavirus Is a Hemagglutinin Recognizing 9-O-Acetylated Sialic Acid as a Receptor Determinant

> **"Viruses and cells.** Strain L-9 of BCV was obtained from R.Rott (Giessen, Germany). **MDCK I cells, a subline of Madin-Darby canine kidney cells,** were maintained as described previously (6).

> **Growth and purification of virus.** BCV was grown in MDCK I cells as reported recently (20). **Virus was harvested from the supernatant of infected MDCK I cells 48 h postinfection.** After clarification of the medium by low-speed centrifugation (2,000 x g, 10 min), virus was sedimented by ultracentrifugation at 112,000 x g for 1 h. **The pellet was resuspended in phosphate-buffered saline (PBS) and layered on a sucrose gradient (5 to 50% [wt/wt] in PBS).** After centrifugation at 148,000 x g for 40 min, the virus band was collected, **diluted with PBS,** and sedimented under the same centrifugation conditions. **The virus pellet was resuspended in PBS and used for purification of the viral glycoproteins.**

Isolation and purification of viral glycoproteins. Viral glycoproteins were **isolated by treatment with n-octylglucopyranoside** and purified by sucrose-gradient centrifugation as described recently (21)."

"Because of the efficiency of S
protein in recognizing Neu5,9Ac2-containing receptors, **it is reasonable to assume that the primary attachment of these coronaviruses is mediated by S protein rather than by HE. This conclusion is in accord with the electron microscopic observation of the viral glycoproteins.** They are visible as a double fringe of projections on the virion surface (4). HE protein, which is smaller in size, forms the inner layer of projections. The peplomers, which are characteristic of coronaviruses, form the outer layer of spikes and are made up of S protein. **Thus, whenever a virus particle approaches a cell, the cellular receptors will first encounter S protein.** For coronaviruses that lack an HE protein, such as avian infectious bronchitis virus, it has been shown that S is the attachment protein (1). **From the results presented here, we propose that attachment of all coronaviruses to cell surfaces is mediated by S protein irrespective of the type of receptors recognized."**

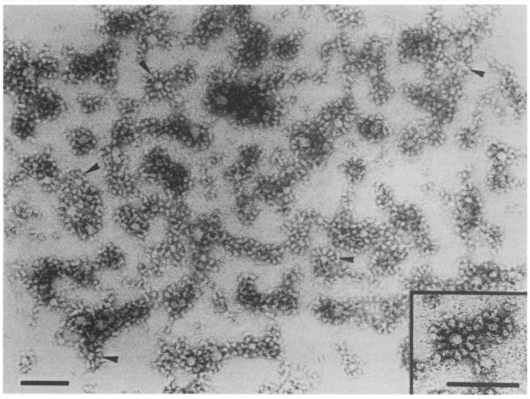
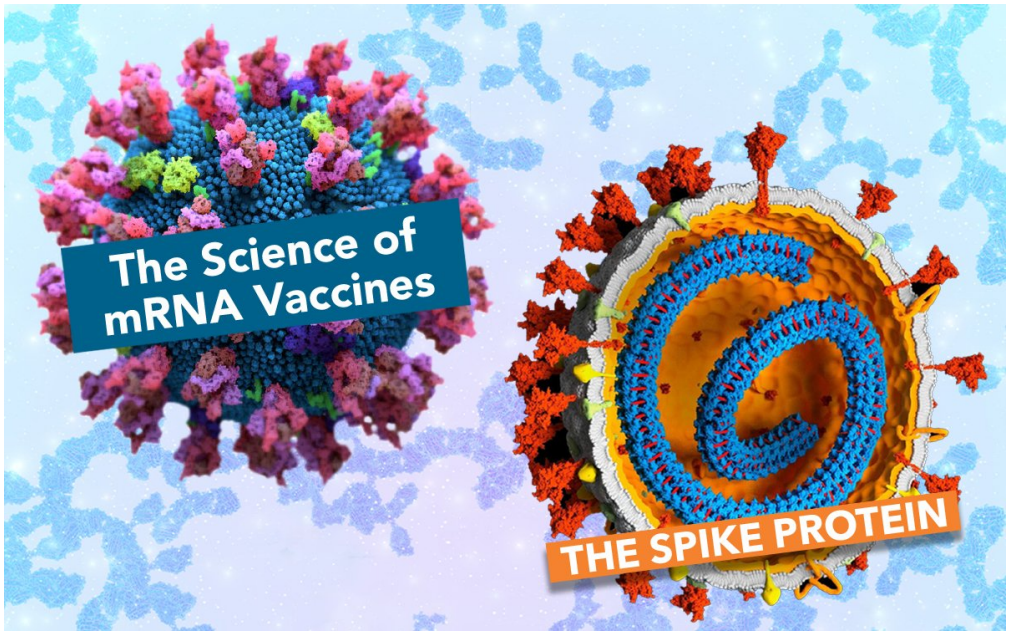


FIG. 3. Electron micrograph of S protein from BCV. The viral glycoprotein was solubilized by octylglucoside and purified by sucrose gradient centrifugation. Before negative staining, the detergent was removed by dialysis. Magnification, ×120,000. The inset shows a rosette at a magnification of ×180,000. The bar indicates 10 nm.

Ah, another "rosette" conveniently shaped like a "coronavirus."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC250319/ <

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC250319/>



In Summary:

- Evidence on vaccine effectiveness (VE) against symptomatic illness and severe disease is expected to become available **only when variant-updated vaccines have been introduced into broader use**
- *In other words, they won't know how well their vaccine works until enough of the fearful and gullible roll up their sleeves and take the plunge…SCIENCE!!!*
- Since the first mRNA vaccines, there has been **continuous and substantial "SARS CoV-2 viral" evolution, particularly in the spike (S)**

protein

- These genomic changes in the "virus" have resulted in several VOC that have circulated in waves, **with varying degrees of immune evasion, some of which have resulted in lower VE of existing "COVID-19" vaccines compared to the initial VE against the index "virus"**
- The magnitude of the reduction in vaccine effectiveness **varies** by:
    1. Product
    2. Schedule
    3. Disease outcome
    4. Variant of concern (VOC)
    5. Time since last dose
- Protection against infection and symptomatic illness due to the Omicron variant is **lower than other variants and declines rapidly, even after a third (booster) dose**
- Those at highest risk for severe disease, hospitalization, and death remain:
    1. Older persons
    2. Those with comorbidities and immunocompromising conditions
    3. Other vulnerable populations
- *In other words, the vaccines have changed absolutely nothing as the 99% of the people who were not at risk are still not and those who were at risk of disease remain so*
- According to the CDC, mRNA vaccines **use mRNA created in a laboratory** to teach our cells *(because the body is apparently stupid)* how to make a protein—or even just a piece of a protein—that triggers an immune response inside our bodies
- That immune response, which produces antibodies, is **what helps protect us from getting sick from that germ in the future** *(except that it doesn't and people still get sick after "infection")*
- The process is outlined as such:
    1. After vaccination, the mRNA will enter the muscle cells and once inside, **they use the cells' machinery to produce a harmless piece of what is called the spike protein**
    2. After the protein piece is made, **our cells break down the mRNA and remove it** *(which is assumed but never observed)*
    3. Next, **our cells display the spike protein piece on their surface** and our immune system recognizes that the protein does not belong there
    4. At the end of the process, our bodies have learned how to help **protect against future infection with the "virus that causes**

COVID-19" *(except that it doesn't as vaccinated people are continually "re-infected")*

5. Any **side effects** from getting the vaccine **are normal signs** the body is building protection *(i.e. side effects are a normal sign that the body has been poisoned and is working to rid itself of the poison)*

- "Coronaviruses" are frequently **claimed** to have a characteristic morphology, **including the possession of a "club-shaped" surface projection or spike (S) glycoprotein**
- However, in common with other aspects of the "coronaviruses," the group **exhibits variation with respect to the shape, size, and distribution of the S protein** on the "virion" surface
- Dimensions of S vary not only among the "coronaviruses" **but also depending on the staining procedure**
- Purification of the S protein of MHV, IBV, HCV strain 229E, bovine "coronavirus" (BCV), and porcine hemagglutinating encephalomyelitis "virus" (HEV) was said to be achieved **using a combination of nonionic detergent and sucrose gradient centrifugation and by affinity chromatography**
- In the initial spike protein purification/isolation paper from 1980 listed by David Cavanagh, we can see:
    - The reasons for the apparent diversity in the polypeptide patterns of "coronaviruses" **are not fully understood**
    - **Differences in technique and imperfect discrimination** of "virion" polypeptides **from those of host origin** may account for some of the disparities
    - However, **even when the same gel systems and conditions are employed in the same laboratory, significant differences** in the number and size of 'virion" polypeptides of different "coronavirus" strains **have been observed**
    - A spontaneously transformed derivative of the BALB/c 3T3 cell line, designated 17 Cl 1, and the L2 derivation of the L929 cell line **were grown in Dulbecco medium supplemented with 10% unheated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 ,ug/ml)**
    - The A59. strain of mouse hepatitis "virus" **was produced in 17 Cl 1 cells**
    - 17 Cl 1 cell monolayers in glass roller bottles were inoculated with A59 "virus" at a multiplicity of 1 to 10 PFU/cell and after an adsorption period of 1 h at 37°C, **50 ml of Eagle minimal essential**

medium with 10% unheated fetal bovine serum was added to each roller bottle and the cells were incubated at 37˚C

- Released “virus” was usually harvested 24 to 26 h after inoculation, after several cycles of infection, when high yields were obtained **and well before significant amounts of cytopathic changes such as cell fusion or lysis had occurred** *(i.e. they did not look for the required CPE/lysis to identify if any “virus” was present as it was assumed to be in there)*
- The “virus” was precipitated **by the addition of 5.0 g of NaCl** per 150 ml of clarified supernatant **followed by a half volume of 30% polyethylene glycol** to give a final concentration of 10% polyethylene glycol and 2.2% NaCl
- After multiple centrifugation steps using various chemicals, radiolabeled compounds **were added to the medium in final** concentration of 2 to 4 uCi of L-3H-amino acid mixture, or [5-3H]uridine per ml, 3 uCi of [6-3H]fucose per ml, and 1 to 4 uCi of [35S]methionine per ml
- “Purified virions” from three or fewer roller bottles were usually pelleted **and resuspended in 1 to 2 ml of TMEN 6 buffer at 4˚C**
- **NP40,** kindly provided by Shell, Inc., **was added to a final concentration of 0.25 to 1%,** and the mixture was shaken vigorously by hand at least 20 times
- The detergent-treated “virions” were then layered at 4˚C over 15 to 50% sucrose gradients **in TMEN 6 buffer containing 0.1% NP40 or at 10˚C over 30 to 75% sucrose gradients in TMEN 6 buffer containing 0.1% NP40**
- Continuous 20 to 50% gradients of **Renografin-76 containing 0.1% NP40** were employed for dissociation of nucleocapsid complexes
- For gel electrophoresis used to separate the proteins, each well was loaded with approximately 40 ul of a radiolabeled sample which had been heated to 37˚C for 15 min **with an equal volume of sample treatment mixture composed of 6 M urea, 4% SDS, 0.05% bromophenol blue in 0.0625 M Tris-chloride, pH 6.7**
- Gels were **run at 125 V for about 4 h under constant voltage** from a Savant power supply
- Gels were **impregnated with PPO (2,5-diphenyloxazole; Sigma) in dimethyl sulfoxide** by the method of Bonner and Laskey, **dried with a Savant gel drier** onto Whatman no. 17 chromatography paper, **exposed to Kodak XR-5 film at -70˚C for 1 to 4 weeks,** and developed with Kodak X-ray chemicals

    - "Purified, concentrated A59 virus" was **disrupted with 1% NP40** and frozen at -75˚C in aliquots
    - A 0.1-ml amount of the "virus" preparation **was inoculated with complete Freund adjuvant** into rabbit footpads, followed after 2 weeks with a second footpad injection **of an additional 0.1 ml with incomplete Freund adjuvant** (Difco) and 1 week later by an intravenous injection of another 0.4 ml **of detergent-treated** "virus"
    - *"There are two types of Freund's adjuvant: complete and incomplete. Complete Freund's Adjuvant, or CFA, is a water in oil emulsion, **which also contains inactivated mycobacteria** (Mycobacterium tuberculosis is most frequently used). Incomplete Freund's Adjuvant, or IFA, **is the same water in oil emulsion,** but does not contain the mycobacteria pathogen."* **https://prosci-services.com/antibody-development-guide/freunds-adjuvant/ < https://prosci-services.com/antibody-development-guide/freunds-adjuvant/>**
    - After the third injection, rabbits were bled from the ear at 1-week intervals **for 1 month**
    - For immunoprecipitates of radiolabeled "viral" polypeptides, a 25-ul amount of rabbit serum **was incubated with 25 to 200 ul of radiolabeled sample in phosphate-buffered saline containing 0.1% NP40 at 0˚C for 1 h**
    - Antigen-antibody complexes and antibody were precipitated **with an excess of purified, Formalin-fixed staphylococci** (Cowan 1 strain) for 10 min at 4˚C, pelleted at 3,000 rpm for 10 min, **washed three times in 1 ml of 0.05% NP40 with phosphate-buffered saline** and then solubilized **in sample treatment mixture by boiling for 1 min** or treatment at 37˚C for 15-30 min
- *Do not feel bad if much of the previous outline went over your head as it is shared in order to show the numerous procedures, manipulations, and alterations the sample must go through to get the desired result*
- In the 1981 purification study, the "virus" once again was a creation from the cell culture process:
    - MHV3 was **grown in confluent secondary mouse embryonic fibroblasts**
    - Monolayers were infected at an input multiplicity of 0.1 infectious particles per cell and following an adsorption period of 1.5 hours at 37 ˚ C, were **incubated for 72 hours at 37 ˚ C in Eagle's MEM with 2 percent foetal calf serum**

    - Aliquots of this “virus” suspension were stored at –70˚C and **used for the preparation of “purified virus” particles and subcomponents**
    - The “virus” was pelleted at 75,000 × g for 1 hour and then **resuspended in 1 ml Dulbecco’s phosphate buffered saline “A” (PBSA)**
- In the 1983 study, the “virus” is another cell-cultured creation:
    - The “virus” was grown in the **chorioallantoic membrane (CAM) cells of de-embryonated chicken eggs**
    - It is stated that there is **less agreement on the composition of the S protein**
    - The **presumptive** S polypeptides of all “coronaviruses” examined were said to have mol. wt. greater than that of the N polypeptide
    - Radiolabelled IBV-M41 was prepared in pairs of de-embryonated eggs; **each egg received 125 uCi [35S]methionine (sp. act. > 800 Ci/mmol) or [35S]methionine plus 165 uCi of a mixture of 15 3H-labelled amino acids**
    - Unlabelled “virus” was **grown in batches of 200 11-day-old embryonated eggs** which were inoculated with approx. 3-5 log10 median ciliostatic dose50 of IBV-M41
    - After centrifugation, the pellet was resuspended **in NET buffer (100 mM- NaC1, 1 mM-EDTA, 10 mM-Tris-HCl pH 7.4)** to a vol. of 20 ml and sonicated at maximum amplitude for 10 s with the 3 mm probe of an MSE **ultrasonic disintegrator**
    - Unlabelled markers used were **phosphorylase b, bovine serum albumin and carbonic anhydrase;** some phosphorylase **was pre-stained with Drimarine brilliant blue K-BL** and the apparent mol. wt. was 110K
    - These studies were said to show that the peplomers of IBV comprise two glycopolypeptides of 90K and 84K in equimolar proportion while the polypeptides of 110K and 75K, variably detected in “virus” preparations, **are probably host polypeptides** *(in other words, they could not separate out the host material)*
- In 1985, a study was done attempting to purify cell cultured “virus” by way of affinity chromatography:
    - The Massachusetts M41 strain of IBV was **grown in the allantoic cavities of 11-day-old embryonated chicken eggs** and “purified” on isopycnic sucrose gradients as described by Cavanagh (1981)
    - ‘Purified virus” was pelleted in a 6 X 14 ml rotor at 70,000 X g for 3 h at 4˚C and **resuspended iphosphate-buffered saline (PBS)**

- An **equal volume of PBS containing 4% (wt./vol.) NP40 was added,** mixed using a Dounce homogeniser and incubated for 2 h at 25˚C
- The immunoadsorbent was **stored in PBS containing 0.2% NaN3 at 4˚C until used**
- It was washed twice **with 3 M NH4SCN in PBS containing 0.1% octylglucoside, four times with PBS and twice with PBS containing 2% NP40** before use
- The solubilised "virus" preparation **was mixed with the immunoadsorbent for 16 h at 4˚C** using a rotary stirrer
- The gel was poured into a chromatography column **and washed with PBS containing 0.1% NP40 (40 ml) and PBS containing 0.1% octylglucoside (10 ml)**
- 3 M NH,SCN in PBS containing 0.1% octylglucoside **was added** and 10 fractions of 1 ml collected
- Those fractions from gel electrophoresis containing detectable "viral" protein **were pooled** and constituted the "purified" protein preparation
- There were other stained bands present, but these are **artifacts sometimes observed, even in the absence of protein, with this staining procedure**
- This paper describes the application of affinity chromatography **using monoclonal antibodies** for the "purification" of the two "viral" structural proteins present at the surface of the IB "virion" – spike and membrane
- IBV was **solubilised in NP40 detergent** and centrifuged in a sucrose gradient **containing this detergent** in order to "purify" the nucleocapsid protein
- The **addition of 1 M NaCl to the sucrose solutions** was required for the "purification" of the spike and membrane proteins, **as they co-migrated in gradients containing low salt concentrations**
- However, the nucleocapsid protein **could not be purified in gradients containing high salt concentrations**
- In other studies (Cavanagh, 1984) "purified" spike material contained some nucleocapsid protein and the membrane preparation **contained other proteins which were thought to be of cellular origin**
- It is stated that there are a number of advantages in using affinity chromatography and that **by making use of the specificity of the**

antibody pure material can be isolated, **even from a crude mixture of proteins**
    - *In other words, they used theoretical entities to stain the proteins and claimed those were the ones they wanted from within a crude mixture of other proteins…i.e. not purified/isolated at all*
- In the first of two studies from 1990, we again get a cell cultured "virus" grown in DBT cells, a mouse brain tumor cells transformed by Rous Sarcoma "virus:"
    - The A59 strain of MHV (MHV-A59), obtained from the American Type Culture Collection, was plaque-purified twice and **passaged four times** at a multiplicity of infection (MOl) of O.Ol **on DBT cells**
    - "Virus" was produced as described previously **in culture medium containing 1 % (v/v) FCS** *(fetal calf serum)*
    - Cell debris were pelleted and "virus" concentrated by precipitation **with 10% (w/v) polyethyleneglycol in O.S M NaCl**
    - "Viral" antigens were resuspended and **dialyzed against TMEN buffer (0.1 M Tris-acid-maleate, pH 6.2, 0.1 M NaC!, 1 mM EDTA),** and kept at -70˚C until used
    - In some experiments, "virus" was labeled **by adding 4 mCi of [3SS]methionine to culture medium at 6 hrs post-infection**
    - For affinity chromatography, concentrated "virus" was **solubilized with 2% (v/v) Nonidet P-40 (NP-40) for 2 hrs at room temperature** (RT), and soluble proteins were **mixed with the 7-lOA-Sepharose gel and incubated** end-over-end for 16 hrs at 4˚C
    - The gel specificity was determined by immunoadsorption of radiolabeled antigen, **extensive washing with 0.1 % (v/v) NP-40 (in 0.2 M phosphate buffer, pH 6.2, 0.1 M NaC!, 1mM EDTA), and elution of adsorbed proteins into electrophoresis sample buffer**
    - Fractions containing "purified" E2/S **were pooled** and used for immunological studies
    - The E2/S glycoprotein used for immunogenicity studies was "purified" from "viral" antigens **concentrated from 1.8 liters of culture medium from MHV -A59-infected DBT cells**
    - The dimeric and monomeric forms of E2/S were "purified" without detectable contamination from other "viral" proteins, **however, a contaminant which was probably of cellular origin, was reproducibly observed**
- In the second study from 1990, the nose swab from a pig was grown in MDCK cells from a dog:

    - "Purified" HEV was **incubated with 3H- DFP for 30 min at 4˚C** and then analyzed by SDS-polyacrylamide gel electrophoresis
    - Following **staining with Coomassie Brilliant Blue** the "viral" proteins N, M, S, and HE became visible
    - In order to isolate the esterase of HEV from the "viral" membrane, "purified virions" **were treated with 1% octylglucoside (OG)**
    - The nucleocapsid as well as the M-protein were pelleted by centrifugation for 30 min at 25.000 x g and the glycoproteins remaining in the supernatant (S and HE) **were loaded onto a 10-30% sucrose gradient in PBS containing 1% OG**
    - Following centrifugation at 42.000 rpm for 16 h in SW55 rotor, fractions were collected from the bottom of the tube and **analyzed by SDS-polyacrylamide gel electrophoresis**
    - S-protein was said to be **detected** in fraction 3, while most of HE was recovered from fraction 6
- In the last study from 1991, BCV was cultured and grown in MDCK I cells, a subline of Madin-Darby canine kidney cells:
    - "Virus" was harvested **from the supernatant of infected MDCK I cells** 48 h postinfection
    - After clarification of the medium by low-speed centrifugation (2,000 x g, 10 min), "virus" was sedimented by ultracentrifugation at 112,000 x g for 1 h and the pellet **was resuspended in phosphate-buffered saline (PBS) and layered on a sucrose gradient (5 to 50% [wt/wt] in PBS)**
    - After centrifugation at 148,000 x g for 40 min, the assumed "virus" band was collected, **diluted with PBS,** and sedimented under the same centrifugation conditions
    - The "virus" pellet was **resuspended in PBS and used for purification of the "viral" glycoproteins**
    - "Viral" glycoproteins were isolated **by treatment with n-octylglucopyranoside** and "purified" by sucrose-gradient centrifugation
    - The researchers state that because of the efficiency of S protein in recognizing Neu5,9Ac2-containing receptors, **it is reasonable to assume** that the primary attachment of these "coronaviruses" is mediated by S protein rather than by HE
    - This conclusion **was in accord with the electron microscopic observation** of the "viral" glycoproteins
    - *In other words, because the S protein was said to be the longer spikes in the EM images, it can be assumed to be used to attach to cells to*

infect them

- Thus, whenever a “virus” particle approaches a cell, **the cellular receptors will first encounter S protein**
- From the results presented here, they proposed that the attachment of all “coronaviruses” to cell surfaces **is mediated by S protein irrespective of the type of receptors recognized**

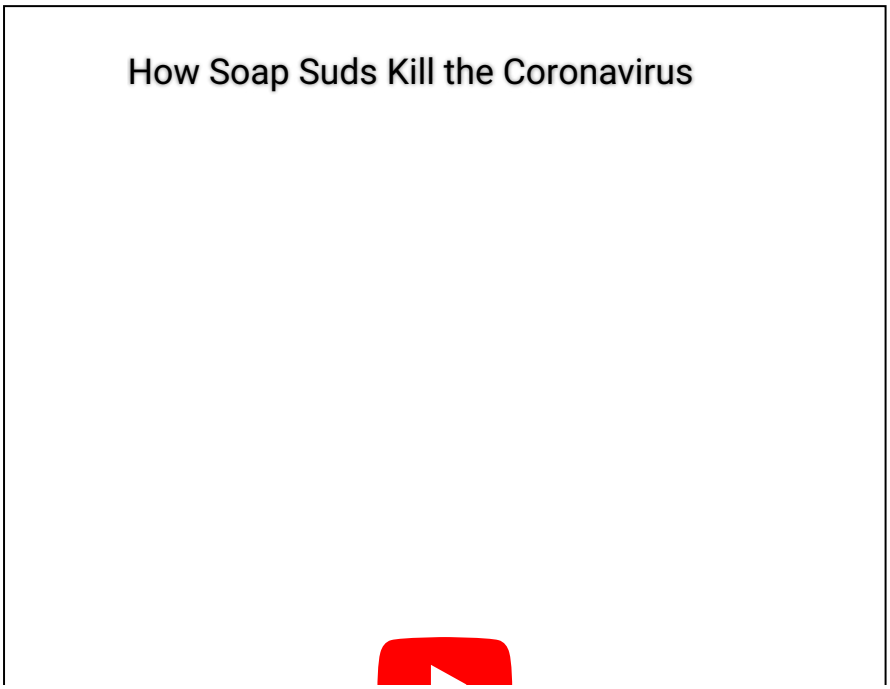
The smaller the particles get, the more impossible it is to purify and isolate.

How far away removed from reality does a substance need to get before any information gained from it becomes utterly meaningless? In the case of any biological sample, is it the moment the fluid is removed from the living organism in order to be studied in a lab under artificial conditions? Is it the moment that the sample is subjected to unnatural chemicals claimed to keep it alive? Is it when the sample is mixed with foreign genetic materials from other species which it would never come into contact with inside the living organism? Is it when the sample is subjected to unnatural g-forces it would never encounter as it is spun numerous times through ultracentrifugation in order to separate its components? Is it the moment the sample is chemically fixed, dehydrated with ethanol, stained with heavy metals, encased in resin, and blasted with electrons? Or is it the moment the sample is added to a gel and electrocuted for 4 hours until the particles are said to separate? At what point does the biological sample lose itself and become nothing but an illustration in a book?

Concerning the “virus” and its assumed subcomponents including the S protein, the invisible particles are subjected to numerous processes that damage and alter the sample in unmeasurable ways. These include but are not limited to:

1. The artificial chemicals and conditions encountered during cell culturing the supposed “virus”
2. The unnatural forces applied to the sample during any purification procedures
3. The various altercations done during electron microscopy preparation
4. The high voltages the sample is subjected to during gel electrophoresis

If you do not think that these processes would damage and alter the biological sample with the assumed “virus” beyond recognition to the point that it loses all biological relevance, you obviously do not know how soap is said to destroy “virus” particles and need to take 2 minutes out of your day to watch the short clip below:



Now that you know how hand soap completely destroys the “virus” from this beautifully animated clip, ask yourself how the “virus” and its subcomponents, including the spike protein, supposedly survive the bombardment of added

chemicals, compounds, forces, etc. in order to be studied. How do the remnants of particles left after these processes hold any significance to what goes on inside a living organism? How can the assumed “viral” particles within a mixed sample containing foreign genetic material from numerous sources, which are then broken into many smaller intermixed particles of the same size, density, and shape, even be claimed to be coming from the same “viral” source?

It’s a 100% guarantee that any living organism would not survive nor remain in the same state it was in if put through similar procedures that these “viral” samples are subjected to. Once you realize this, and once you know that the researchers have admitted numerous times that the current technology not only damages the materials but can also not purify and isolate particles of the same size, shape, and density away from each other, it becomes clear that any information gleaned from these heavily altered and mixed populations hold no significance whatsoever. At best, the particles created serve only as an illustration. A work of twisted art created in a lab. The picture used to sell the story, the fear, and the vaccine. The “coronavirus” and the “spike protein” are just the latest marketing mascots successfully utilized for yet another profitable fear campaign.