COVID-19

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Much has been said in the popular press about the world-wide COrnaVIrus Disease 2019 (COVID-19) pandemic. Some of this information is accurate, some is exaggerated. In some cases vital information has not been presented, while other information is simply wrong. As business activity resumes in the U.S. and other countries, occupational health and safety personnel will have to make some extremely difficult decisions. Those decisions will have to be based on a thorough understanding of the scientific facts and not fear, partial information or distortions. So, what are the facts? What is a coronavirus? How does it work? How did it get that name? Where did it come from? How does it spread? Is there any way to stop it? Do masks work? What about social distancing, hand sanitizers and hand washing? What do the coronavirus statistics mean? Can a vaccine be made? Does post-infection therapy work, and is it safe? Does vitamin C and zinc do anything, or is it just a hoax? These are some of the questions that will be addressed in this article.

The Beginning of Life on Earth [Ref. 1]:

Sixty years ago it was thought that perhaps viruses were a link between the lifeless Earth of 4.5 billion years ago and the first cells that appeared about 3 billion years ago. However, today it seems that this notion is only partially true.

The operation of complex *eukaryotic* life (everything from amoebas and algae to man) is based primarily on two great biochemical pillars. First, there are large complex protein molecules (e.g. *enzymes*, or catalysts) that might be thought of as macromolecular micro-machines to help carry out the processes of life. Proteins are very complex, but also very specialized for their tasks, and were eventually favored by evolution. For example, the protein myoglobin (a constituent of hemoglobin, necessary for respiration in higher animals) is built from 153 amino acids, and there are 20 types of amino acids.

The second pillar is the deoxyribonucleic acid (DNA) molecule, that may be thought of as a complete set of instructions for building and maintaining living cells, including those macromolecular proteins. Instructions are encoded via a sequence of four *nucleotides* (adenine [A], cytosine [C], guanine [G], and thymine [T]).

By contrast, the macromolecular machinery of the first life on Earth appears *not* to have been based on proteins and DNA, but completely on more problematic ribonucleic acid (RNA, made up of a sequence of A, C, G, and another nucleotide called uracil [U]). The evidence for this astonishing statement comes from cells themselves. Eukaryotic cells contain relics from the ancient RNA world. These relics include *messenger RNA* (called mRNA) that is a blueprint (copied from a very small part of the DNA within the cell's *nucleus*) for building proteins, and *transfer RNA* (called tRNA) that actually assembles the blueprinted proteins on *ribosomes*, molecular "work-benches" made (at least partially) of *ribosomal RNA* (rRNA). Life needed to conserve these cumbersome archaic RNA structures because, in the beginning, there were no versatile protein micro-machines available to build still more proteins.

It is suspected that some viruses, like the coronavirus, have descended from a very ancient line because they contain the instructions for building daughter virus particles in the form of RNA (called *vRNA*), while others viruses are clearly more modern inventions of evolution because their instructions are in the form of DNA (like ordinary eukaryotic animal cells). In either case, *all* viruses are parasitic and depend on the energy and replication machinery of complex eukaryotic cells to multiply. So, it appears that viruses may have co-evolved with cells since the very beginning of life on Earth!

The Structure of Members of the Genus Coronavirus [Ref. 2]:

There are many types of simple infectious particles. There are prions, infectious proteins that are capable of polymorphing other proteins. There are short pieces of naked RNA only about 300 nucleotides long (called viroids) that can cause disease. There are also small "satellite viruses" which depend on other viruses for their replication.

True viruses, however, should be thought of as complex infectious particles. They consist basically of a genome (RNA or DNA) within a protective shell. The smallest genomes contain only about 3000 nucleotides. At the high end, the largest known RNA viruses, the coronaviruses (Fig. 1), have genomes containing 20,000 to 30,000 nucleotides. DNA viruses can be even bigger! By comparison, bacterial genomes can contain 4,000,000 nucleotides, and the human genome contains something

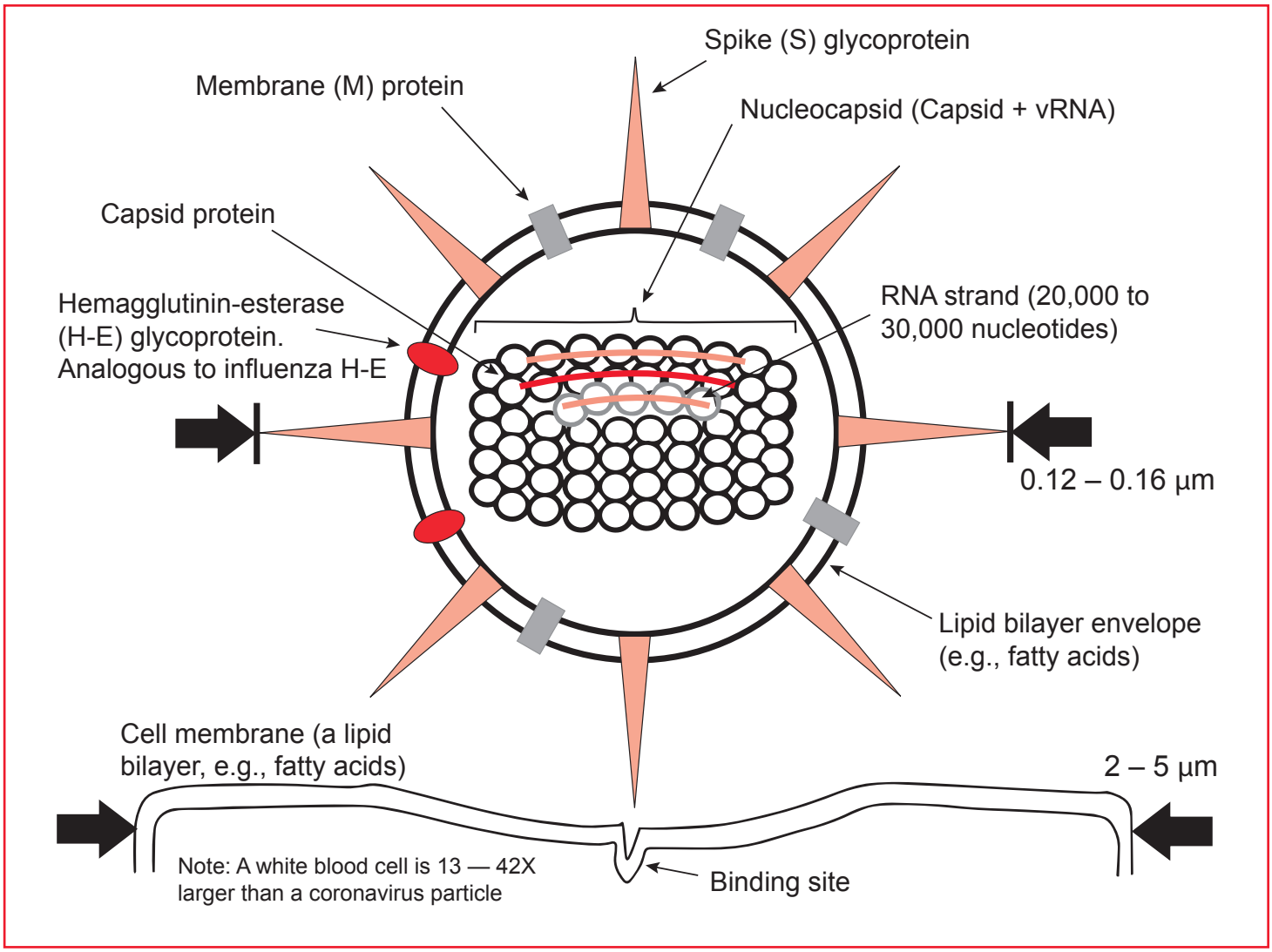

Figure 1 – Coronavirus "cartoon" (model) attacking a cell.

on the order of 4 billion nucleotides. The genome of RNA viruses is encoded for the production of a molecule called *replicase* (or RNA polymerase). Basically, replicase is a macromolecular micro-machine capable of copying the viral genome from the infected host cell's A, C, G, and U. Because there is no proofreading mechanism during RNA synthesis, there is roughly an error rate of one nucleotide for every 10,000 copied. Therefore, the coronavirus genome is just about as large as possible for RNA viruses in general if the genome is expected to be copied with reasonable fidelity. Each coronavirus replication can be expected to have two or three genetic misspellings (sequence errors). Although that might not be enough to render the mature extracellular virus particle (*virion*) non-viable, it does give the coronavirus a way to avoid a host's immune system by mutating to a different unrecognizable strain.

So, there is a distinct evolutionary advantage for a simple parasite like the coronavirus to remain (at least genetically) in the unstable RNA world. For complex organisms like birds, mice, or humans, with their much larger genomes, evolution favors DNA with an error rate about one million times *lower* than RNA during replication. That makes DNA a stable repository for genetic information.

The next major component of a virion is the *capsid*, a protein shell that encloses and protects the genome from the environment when it is outside a cell. The coronaviruses have a helical (corkscrew-like) capsid that is 10 to 20 nm in diameter. Nature prefers helical capsids because the genome that is contained inside can grow over evolutionary time (as the coronavirus genome has) and still be protected by just adding successive turns to the helical structure, making the bottle a little longer. Proteins must be used for the capsid because viral parasites hijack *cellular* machinery to build more virions, and cells ultimately read viral RNA (which, in the case of the coronavirus, is basically an infectious *messenger* RNA) to make *proteins* (viral proteins in this case) that are capable of self-assembly

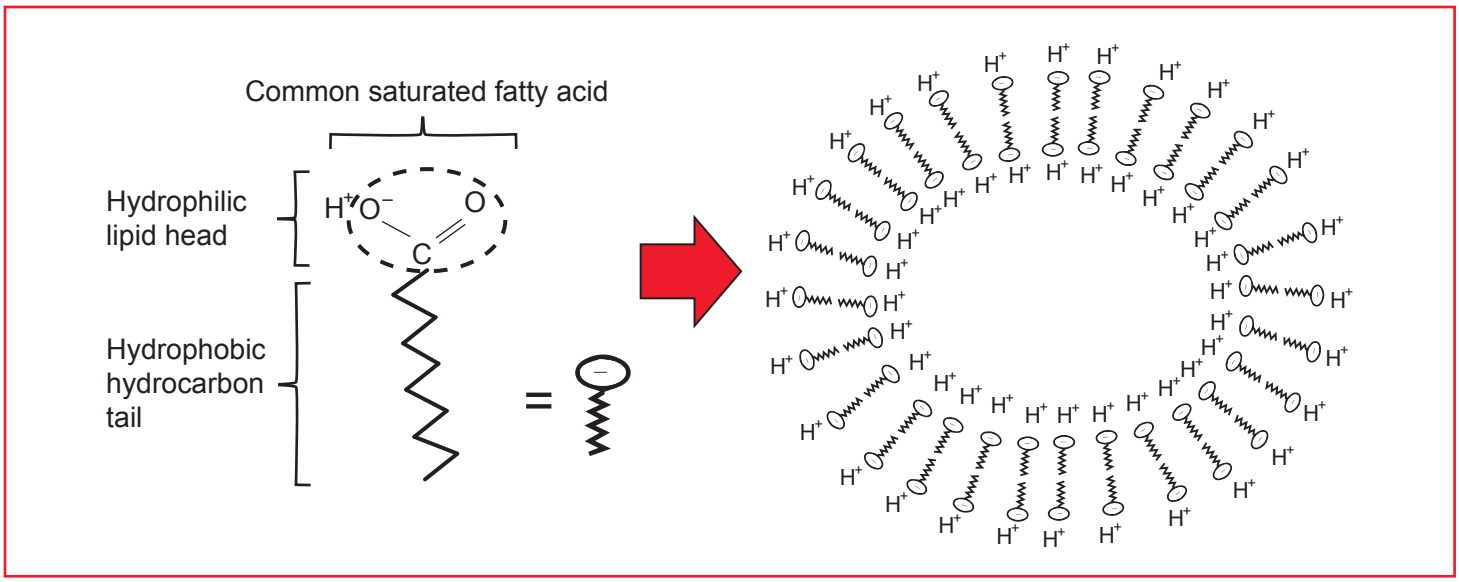

Figure 2- The structure of a lipid bilayer bubble: Note the arrangement of lipid heads and tails. If this fatty bubble contained a nucleocapsid, it would be called a viral envelope, provided the bilayer contained certain prerequisite glycoproteins. *The outer membrane of animal cells has a similar structure, as do the membranes of organelles (structures) within a cell. A bubble of lipid molecules is stable under the slightly acidic conditions (pH 5 → 7) found on the interior of* liposomes. *This is a very important point that will be returned to later when liposomes are discussed in the section on "Post Infection Pharmaceuticals."*

around a strand of RNA from solution. The daughter capsid plus the vRNA is called a *nucleocapsid* (Fig. 1).

Surrounding the nucleocapsid is the *lipid envelope* (Fig. 1). It primarily consists of a double layer of lipid molecules, and each rod-like lipid molecule has a polar hydrophilic (water-loving) head and a long, more or less straight, hydrophobic (greasy, water hating) hydrocarbon tail. The tails of molecules in one of the two layers point to the tails of molecules in the other layer so that the hydrophilic heads can face water both outside and inside the bubble-like envelope. Weak Van der Waals forces between the hydrocarbon tails of a lipid molecule's lateral neighbors keeps them all standing straight like people in an over-packed subway car (Fig. 2). The membranes of living cells also have the same structure as that shown in Figure 2. Therefore, the naked nucleocapsid of a daughter virion in an infected cell can create an envelope around itself by *budding* through one of its host cell's internal membranes (an intracytoplasmic membrane), becoming a fully functional virion capable of infecting other cells. However, finding a suitable host cell to infect is not so easy, unless you have suitable biochemical "eyes."

Imbedded within the virion's envelope are *glycoproteins* (proteins with sugar molecules attached — Figure 1). All coronaviruses have at least two types: a spike protein (S) and a membrane protein (M). The S protein projects above the lipid bilayer and produces the characteristic corona (Latin for crown) around the envelope — hence the genus name. Furthermore, it is these spike proteins that possess the receptor binding activity and act like biochemical eyes allowing the parasite to identify its host cell prey. The M protein (called an *integral protein*) spans the lipid bilayer and only slightly protrudes beyond the envelope. Some coronaviruses possess a third, and very surprising, envelope glycoprotein called hemagglutinin-esterase (or H-E for short). H-E allows the corona virion to bind to and agglutinate red blood cells. Astonishingly, all influenza C viruses possess a similar (*homologous*) protein! It is thought that some coronaviruses acquired H-E through exchange of genetic material with Influenza C sometime in the past. Phenomena of this kind has led to the speculation that some partial immunity to COVID-19 might be generated by vaccination for other diseases. Although this may be theoretically possible, it seems unlikely because the shared protein might not be similar enough, or in sufficient dose, to elicit a significant immune response. In reality, the chemical composition of the coronavirus envelope is somewhat variable and easily mutates. This *antigenic drift* complicates vaccine development. In spite of all this, it is still a good idea to get a flu vaccination since a compromised immune system opens a person up to secondary infection by coronavirus.

The entire coronavirus assembly is 120 – 160 nm (0.12 – 0.16 μm) in diameter, large for an RNA virus, but needed to accommodate the larger genome of the virion (Fig. 1). By comparison, a human white blood cell is 2 to

5 microns in diameter, about 13 to 42 times larger than a corona virion. So, these infectious particles can still be easily engulfed (*endocytosed*).

Two Fundamental Laws of Microbiology

The two fundamental laws of microbiology are:

1. The Law of Minimum Infectious Dose (MID): This law simply states that *a host must receive a minimum number of infectious particles to start an infection*. For example, Primary Tuberculosis (a lung infection transmitted almost exclusively via respiratory mucus aerosols) requires at least about 10 tubercle bacilli (Mycobacterium tuberculosis) [Ref. 3]. In general, humans are rather easily infected with the bacterium, so that 10 infectious particles might be considered a lower limit for lung infections. Currently, no one knows what the MID is for COVID-19, but the MID for two related infections are thought to range from a few hundred to thousands of virions for normal people. Therefore, a personal protective procedure does NOT have to be 100% effective to prevent disease. Any procedure capable of reducing the initial infective inoculation to a level below the MID is adequate.
2. The Law of Proportional Infectious Dose: This law states that *the magnitude of the initial inoculation is proportional to the magnitude of the symptoms and inversely proportional to the delay time associated with their onset* [Ref. 3]. For example, if a person is infected by n Clostridium tetani (tetanus) rods that produce a concentration C of tetanospasmin neurotoxin, then an infection by 3n rods would produce a neurotoxin concentration of about 3C, and the concentration level C, with its concomitant symptoms, would be reached sooner. Any personal protective equipment or procedure capable of reducing the initial infective inoculation will reduce the symptoms of a COVID-19 infection, and may be life-saving.

Enough background information has now been presented to answer some very practical questions. And, there is no better place to start than to talk about masks.

Do Masks Work?

There has been a great deal of controversy over the effectiveness of masks. Everyone seems to agree that masks can protect others if an infected person should sneeze [Ref. 3]. But, what about the person on the receiving end? If an unmasked infected person sneezes, do others get any protection by wearing a mask? The rules stated previously can answer this question. As an example, consider a person wearing an N95 mask (i.e., one that is capable of stopping 95% of infectious particles from, say, a sneeze). Now, according to the Law of Minimum Infectious Dose, a host would have to intercept at least 200 virions, and in all probability a lot more than that (perhaps 10^3 – 10^4 virions), to start an infection.

In summary, any reduction of the original infective inoculation is helpful since it can minimize the severity of symptoms (Second Law) and, if the inoculation is below the MID, it may even eliminate the possibility of infection altogether (First Law). Furthermore, these notions are supported by clinical [Ref. 4] as well as statistical evidence [Ref. 5]. In countries where the culture or public policy support wearing masks, the average per-capita weekly increase in coronavirus mortality (predictions for May 9, 2020) was almost four times less than in countries that do not. Masks seem to be life savers, but they are not a *perfect* solution. After all, even while wearing a double mask you can smell the tobacco smoke of a nearby smoker. So, something is getting through. *However, the strategy here is* ***reduction*** *of inoculation*.

What About Social Distancing?

The U.S. Center for Disease Control (CDC) guideline is a *minimum* of 6 feet of separation. Let's see if that

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1. The distortion of a liquid droplet in motion relative to an air mass is well known. Meteorologists have studied the problem of the distortion of a water droplet falling in a cloud updraft. As might be suspected, the drop flattens and becomes "pancake-like." Also, the perimeter begins to ripple and the drag force increases. At still greater relative velocities, a drop can break up into even smaller droplets with much shorter ranges. All this means that the range (X) of a distorted droplet is decreased relative to a spherical droplet.
2. The derivation of eq. 1 is left as an exercise for the reader. Start from the equation of motion in differential form

$$(V\text{-}dV)^2 - V^2 = 2\, a_{drag}\, dX.$$

Expand and discard terms of order $(dV)^2$, then substitute Stokes' Law for a_{drag},

$$a_{drag} = F_{drag} / M = 6\, \pi\eta\, R\, V / M.$$

After integration, use the fact that the mass M of a spherical droplet is $(4/3)\, \pi\, R^3\, \rho$, where R and ρ are a drop's radius and density respectively.

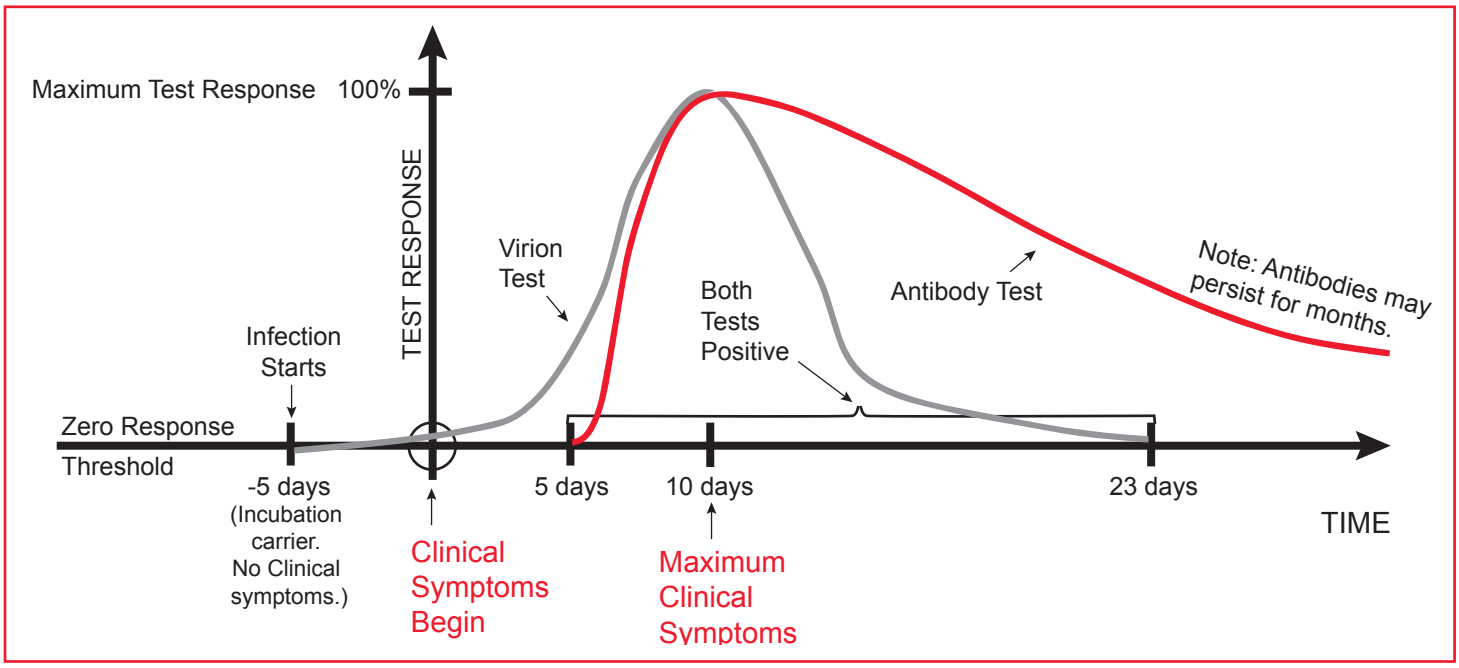

Figure 3 – Comparison of responses from virion and antibody tests vs. clinical symptoms as a function of time [Ref. 10]. The curves are approximate and will vary from one manufacturer to another. Physicians should understand these curves for the tests they are employing.

makes sense. Suppose a spherical infected water droplet is ejected from a person's mouth during a sneeze. How far will the droplet go in *still air* before coming to rest [Ref. 3, Figure 13.15]? It can be shown by integrating one of the equations of motion, using Stokes' Law for the atmospheric drag force — i.e., assuming laminar air flow over a spherical droplet that does not undergo distortion in flight — [Ref. 6][1], that

$$X = (2\,V\,\rho\,R^2) / (9\,\eta)\,, \qquad (1)^2$$

where V is the initial velocity of saliva particles (100 mph or 4470 cm/sec, *maximum*), ρ is the density of water (1 gm/cm^3), R is the radius of the droplet (the geometric mean is about 50 μm), and η is the viscosity of air at 20^{0}C (181 x 10^{-6} dyne sec / cm^2), so that the distance X is about 137.2 cm, or 4.5 ft. Therefore, the CDC "six-foot rule" seems reasonable. Of course, saliva particles with a larger radius and greater possible range are common, but gravity also pulls them down faster. Furthermore, it must be remembered that the calculations above apply to *still air.* If there is a draft due to ventilation or wind, they don't apply, and greater separation is needed to insure safety! It has, unfortunately, been very difficult to get people to follow a six-foot distancing rule, and they are unlikely to voluntarily submit to greater separation.

What About Hand Washing and Sanitizers?

There is a lot more chemistry to washing your hands than you might think. First of all, a molecule of "soap" (detergent) looks like a rod with a polar hydrophilic head and a hydrophobic hydrocarbon tail. In fact, a soap molecule has a structure similar to a lipid molecule (see the previous discussion on the coronavirus envelope). There is a rule in chemistry that may be stated as "like dissolves like." What that means in this case is that the coronavirus envelope will dissolve in soapy water. That envelope, with its binding glycoproteins, is necessary for the corona virion to infect a host cell. Therefore, detergents are extremely destructive to all coronaviruses.

The author has noticed that some bars of soap can become quite hard and rather insoluble as they age. When that happens, the bar of soap should be thrown away. Liquid soaps, of course, don't have this problem. A good lather is important because the bubbles lift particles from the hands in the same way that carbonated water (club soda) lifts dirt and stains from a carpet. Soap also reduces the surface tension of water and allows wetting of virus particles prior to and during flotation. There is also a general rule that hand washing should proceed for *at least* 20 seconds. Here, the emphasis is on the words "*at least.*" Actually, it takes about 60 seconds of interaction for soap to do about all it can.

When washing isn't practical, a hand sanitizer containing ethanol (80% by volume), or isopropanol (75% by volume), together with glycerin (less is better) and 0.125% (by volume) hydrogen peroxide (a powerful *oxidizing agent*) is known to be effective against COVID-19 virions, provided the wipe-down lasts for 30 seconds or

more [Ref. 7]. Other formulations containing even more alcohol have also been suggested [Ref. 8]. In many cases the alcohol content of hand sanitizers is too low for them to do much germ killing of any kind.

For surfaces, wipes soaked in bleach are reliable cleansing agents. And, you can make them yourself if they are not available in a store because of "stockpiling" behavior. Just soak a paper towel in a 10% (by volume) solution of concentrated bleach and water. The wipes can then be stored in a plastic sandwich bag for future use. Crime laboratories use the 10% bleach solution to wipe down all work areas. Surfaces are then just allowed to air dry. The same procedure can be followed in any office or other work space.

There are also iodine-based surgical scrubs for the hands that are very effective. Both chlorine (in bleach) and iodine belong to a group of chemical elements called halogens. And, halogens are powerful *oxidizing agents* (in the "electron capture" sense of that word). Halogens can oxidize (burn) the S (especially), M, or H-E proteins of the coronavirus envelope (discussed in the previous "The Structure of Members of the Genus Coronavirus" section), thereby depriving virus particles of their host recognition and binding function. Any virion that has had its glycoproteins burned by halogens and its lipid bilayer striped by detergent so that it can neither recognize, bind to, or fuse with a host is doomed.

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What About Testing?

It will be assumed that all tests discussed in this section are 100% accurate. The specter of false positives and negatives will be addressed in a succeeding section using a powerful mathematical tool.

When it comes to tests, there are two types. First, there are *virion tests* that test directly for the presence of virions in a patient's nasal passages or throat (*secretory test*). Microbiologists know whether a virion causes COVID-19 by the "spelling" (A, C, G, U sequence) of its RNA genome. The other test is an *antibody test* that involves detection of antibodies in a drop of the patient's blood (*serological test*). The human body develops antibodies in response to invasion by an *antigen*, like a COVID-19 virion.

In either case, clinically active disease may, or may not, be present. If a virion test is positive, but there is no sign of clinical disease, that person is a *carrier*. That is to say, he/she may shed virions, even though they do not feel sick themselves. Usually carriers are in the early stages of disease and will soon become ill (*incubation carriers*). Technically, all individuals infected by COVID-19 are incubation carriers for about five days [Fig. 3]. Note that there are also *convalescent carriers* who can shed microbes (e.g., *Corynebacterium diphtheriae*) for a short time (depending on the disease) after clinical symptoms have passed. The convalescent carrier phase has been observed in COVID-19 patients and may last as long as seven days [Ref. 9]. *Chronic carriers* (e.g., "Typhoid Mary") may continue to harbor an infective agent asymptomatically within their body for years and may spread it to others (not yet observed for COVID-19). On the other hand, if a subject is antibody positive, that just means that they have come in contact with the virion and it has elicited an immune response. If they have no sign of clinical illness, they may have destroyed the infection in a previous encounter. In the latter case, they now have some level of immunity and may not be able to pass on an active infection.

Suppose a subject is antibody positive, virion negative and has no clinical sign of disease. In that case, the subject has probably had the infection and destroyed it, or at least contained it, so that it cannot be transmitted at that time. Suppose the reverse, that a subject is virion positive but antibody negative. The subject has been infected, but has not yet developed antibodies, or the subject has a compromised immune system (i.e., cannot develop antibodies). What about a double positive? As Figure 3 shows, this situation is concomitant with maximum clinical symptoms. Such a person may be very sick and should be in a doctor's care. But, even if symptoms are mild, these patients are still very infectious and should be quarantined. What about a double negative? Such a person has never come in contact with COVID-19 or, if they have, it was long enough ago that they have lost their immunity. Of course, this discussion assumes that the tests employed accurately reflect what

Table 1 — Truth Table for COVID-19 tests (100% test accuracy assumed; N.A. = Not Available)

Virion Test	Antibody Test	Clinical Symptoms	Diagnosis
+	+	+	Needs medical attention for COVID-19 infection.
+	+	-	Contradictory! Antibodies can only appear *after* clinical symptoms. This patient may be asymptomatic but infectious (a carrier).
+	-	+	Infected subject has not yet developed antibodies.
+	-	-	Infected subject has not yet developed antibodies (incubation carrier who will soon become sick).
+	N.A.	+	COVID-19 infected. Needs medical attention.
+	N.A.	-	COVID-19 carrier. Needs medical attention.
-	N.A.	+	Has some condition. If COVID-19, virions can no longer be detected by the test employed.
-	N.A.	-	Clinically healthy, but may be in the very early stages of infection (1st or 2nd day) before any virion detection can be made.
N.A.	+	-	Probably immune from a past COVID-19 infection. Follow up with a virion test
N.A.	+	+	Has come in contact with COVID-19 and has some condition. Follow up with virion test.
N.A.	-	+	Has some condition. Follow up with virion test.
N.A.	-	-	No contact with COVID-19, or is an incubation carrier, or has destroyed an old infection and lost immunity
-	+	-	Has come in contact with COVID-19. Probably immune if antibody test yields *truth*.
-	+	+	Has come in contact with COVID-19 and has some condition. Perhaps virions can no longer be detected by the test employed (check manufacturer's specs)
-	-	+	Probably never exposed to COVID-19, or has destroyed an old infection and lost immunity, but has some condition. COVID-19 improbable.
-	-	-	Probably never exposed to COVID-19, or is an incubation carrier in the very early stages of infection (unlikely), or has destroyed an old infection in the remote past and has lost immunity.

they measure.

The Truth Table (Table 1) summarizes all of the 16 possibilities for *perfect* tests. A *perfect* (100% accurate) test will be defined as one that reveals *truth* (the actual virion/antibody state of a subject). A COVID-19 antibody test can only be positive *after* the onset of clinical symptoms, and there may be a time delay (~5 days) between that onset and the production of antibodies, during which time a clinically ill patient may be antibody negative. Furthermore, it is not clear how long antibodies last after convalescence. Although a virion test can be positive slightly before the onset of clinical symptoms, this is

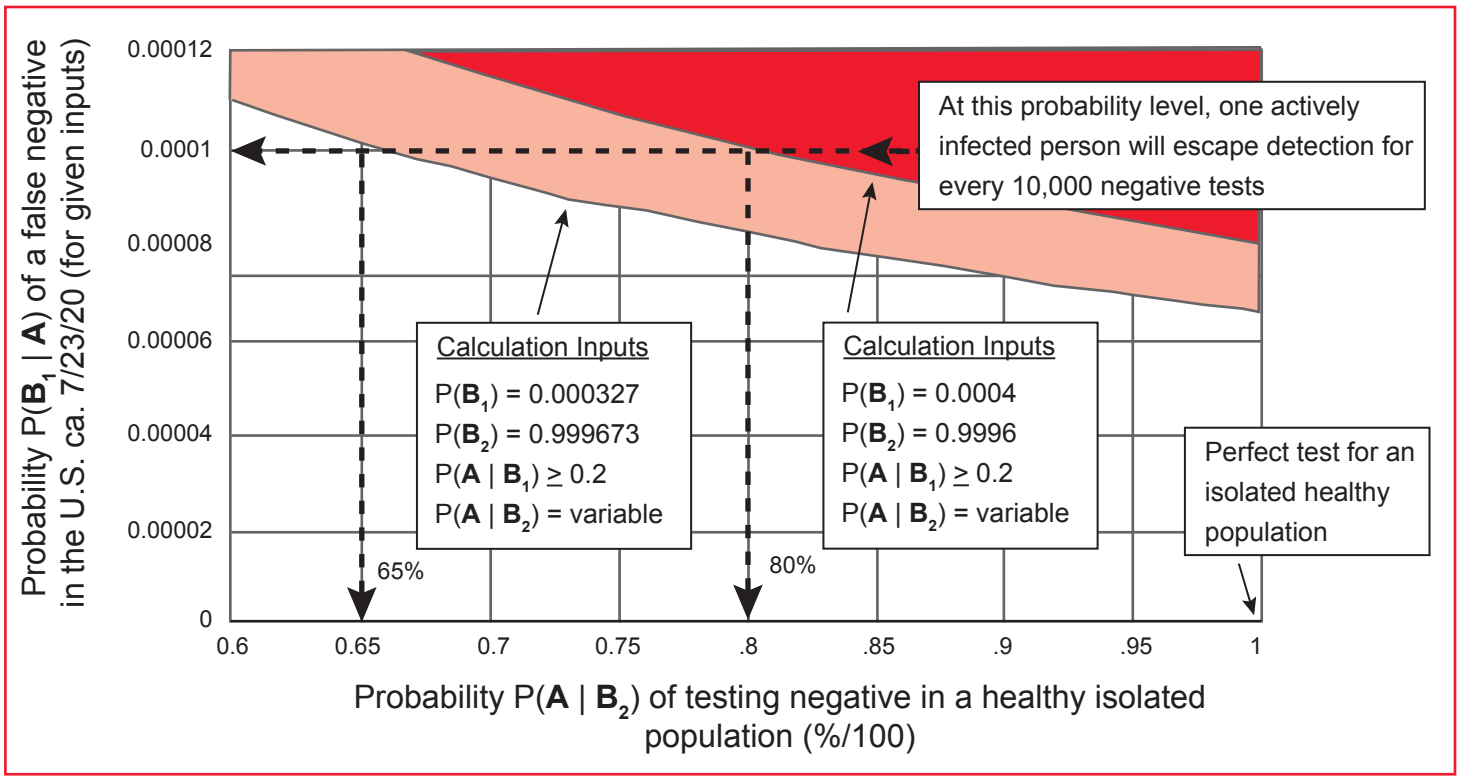

Figure 4 — $P(\mathbf{B}_1 \mid \mathbf{A})$, false negatives, vs. $P(\mathbf{A} \mid \mathbf{B}_2)$, the probability that a test correctly reads negative in a healthy population. According to this graph (at the boundary between the shaded and unshaded areas) a manufacturer must design a test that reads negative 65% of the time in an isolated healthy population to produce false negatives only 0.01% of the time when 0.000327% of the U.S. population is actively infected (shedding virions). The shaded areas (light and dark red) indicate where $P(\mathbf{B}_1 \mid \mathbf{A})$ may lie when $P(\mathbf{A} \mid \mathbf{B}_1)$ is strictly greater than 0.2. Note that the light red area continues on into the dark red area.

usually not seen unless there is a strong suspicion of infection and medical personnel are monitoring the patient daily (or every other day). Usually, a clinically sick person goes to a physician for a diagnosis, and a virion test may reveal active infection. Therefore, it will be assumed that a virion test can be positive only *slightly before* and *after* the onset of clinical symptoms for a period of time that will depend on the test. Tests that violate these rules will not be considered here, nor will the complexities caused by immunity suppression.

Next, consider an imperfect test that is capable of yielding false positives and false negatives that have nothing to do with test timing. There have certainly been complaints along these lines. How should a person respond to this difficulty? Suppose a patient is virion negative, antibody positive, and clinically negative for COVID-19 (13th case in the Truth Table), but has a normal immune system. Then what? Such a patient probably had past contact with COVID-19. However, a physician might also suspect that the antibody test yielded a false positive. How often does that occur? There is a mathematical law called Bayes' Theorem that, for false positives, might be restated in words as follows: *For a **sufficiently rare** disease, and a test that is less than 100% accurate, most positive test results will be false positives.* A numerical example will help clarify this surprising statement, but first some terminology is needed. The *conditional probability* P(1st event | 2nd event) is just the probability that the first event will occur given the *fact* that the 2nd event *has* occurred. To be concrete, consider the skin test for active pulmonary tuberculosis because the statistics for this disease are well known [Ref. 11]. Let event **A** be a "positive TB skin test." Then, either:

$\mathbf{B}_1$ = {"a person really *has* TB"} (**A** was accurate)
$\mathbf{B}_2$ = {"a person *does not have* TB"} (**A** was wrong).

Then Bayes' Theorem states that

$$P(\mathbf{B}_1 \mid \mathbf{A}) = \frac{P(\mathbf{B}_1) \bullet P(\mathbf{A} \mid \mathbf{B}_1)}{P(\mathbf{B}_1) \bullet P(\mathbf{A} \mid \mathbf{B}_1) + P(\mathbf{B}_2) \bullet P(\mathbf{A} \mid \mathbf{B}_2)} \qquad (2)$$

Now, $P(\mathbf{B}_1 \mid \mathbf{A})$ is, of course, exactly what is wanted. It is the probability that the skin test will be "true" given the *fact* that a positive skin test *has* occurred. From clinical history, it is known that a TB skin test will be positive 98% of the time on patients that really *have* TB (i.e., P(**A**

$| B_1) = 0.98$). It is also known that a TB skin test will be *positive* 1% of the time on patients *that do not have* TB (i.e., $P(A | B_2) = 0.01$. From epidemiological statistics it is known that two people out of 10,000 have TB. The rest of the population is free of TB. Therefore, $P(B_1) = 2/10{,}000 = 0.0002$ and $P(B_2) = 1 - 0.0002 = 0.9998$. Substituting these figures into equation 2 yields

$$P(B_1 | A) = (0.0002)(0.98) / [\ (0.0002)(0.98) + (0.9998)(0.01)\ ] = 0.02 \qquad (3)$$

Therefore, for the figures in this example, there is only a 2% chance that a person with a positive skin test for TB actually has the disease. Even though the test is accurate, the disease has been so rare up until very recently that most positives have been false! That is why a positive TB skin test is often followed up by a chest X-ray.

The calculations above can now be applied to our current COVID-19 pandemic; where **A** = "a positive COVID-19 *antibody* test", B_1 = {a person really *has* been infected by COVID-19}, B_2 = {a person *has not* been infected by COVID-19}. On June 11, 2020 the CDC (www.CDC.gov) reported 115,000 total deaths in the U.S. from COVID-19 infection. Since the death rate is about 1%, the original number of *total* clinical infections must have been about 11,500,000. Given that the population of the U.S. is about 330,000,000, $P(B_1) = 11{,}500{,}000/330{,}000{,}000 = 0.035$ and $P(B_2) = 1 - 0.035 = 0.965$.

It will be assumed that once infected, antibodies will remain in the blood regardless of whether the test subject has an active clinical infection at the time of the test or not. The reason for this is that the time interval from March 11, 2020 (approximate start of the epidemic in the U.S.) to June 11, 2020 is only three months. One can now ask, "What is the probability (on June 11, 2020) that a positive antibody test result is accurate?" Repeating the calculation above, using the COVID-19 values for $P(B_1)$ and $P(B_2)$, yields $P(B_1 | A) = 0.78$, or 78% accurate. Quite good in the sense that the remaining doubt can easily be removed, as will be demonstrated. ***But** that "good" result assumes a test manufacturer can produce a test that is 98% accurate (i.e., $P(A | B_1) = 0.98$) when testing patients who are **known** to have been infected (say, hospital patients with clinical COVID-19 infection), **and** that the test gives a false positive only 1% of the time (i.e., $P(A | B_2) = 0.01$) when used on people who are **known** to be free of infection*. It has proven to be very difficult to produce tests with such high fidelity. Currently, there are limited statistics on intrinsic test error. One claim puts $P(A | B_1) = 91\%$ to ~ 95%, while the false positive $P(A | B_2)$ was not specified [Ref. 12]. The COVID calculations here give the reader a sense for the kind of numbers that physicians really need for this pandemic.

"On July 23, 2020 the CDC (www.CDC.gov) reported 1,078 new deaths (24-hour period). If the death rate is about 1% in the U.S., it can be expected that these deaths stemmed from about 107,800 cases, all of whom should have had a positive virion test at some point in time. Therefore, as a *very rough guesstimate,* on the average for U.S. cases, $P(B_1$; virion test) ≈ 107,800/330,000,000 = 0.000327 = 0.0327% (ca. 7/23/20)."

In cases where $P(B_1 | A)$ is high (i.e., close to unity), repetition is a valid way of eliminating false positives. For example, suppose $P(B_1 | A) = 0.78$. Then the probability of a false positive is $P(B_2 | A) = 1 - P(B_1 | A) = 0.22$, and the probability of two false positives in a row is $(0.22)^2 = 0.0484$, or just under 5%. The probability of three false positives is only about 1%. Clearly, as the number of tests increases, the probability of maintaining a sequence of false positives decreases rapidly and approaches zero.

False negatives can also be problematic. The reason for this is simply that a false negative means that an infected individual could be released into the general population. In order to evaluate the impact of false negatives, the calculations above must be repeated, only this time A = "a negative COVID-19 *virion* test" and B_1 = {a person really *has active* COVID-19} and B_2 = {a person *does not have* active COVID-19}. Notice that the word *"active"* has been used in the definitions for B_1 and B_2. This is because virion tests only detect *active* infection. Therefore, $P(B_1$; virion test) << $P(B_1$; antibody test), but it is unclear how much less.

On July 23, 2020 the CDC (www.CDC.gov) reported 1,078 new deaths (24-hour period). If the death rate is about 1% in the U.S., it can be expected that these deaths stemmed from about 107,800 cases, all of whom should have had a positive virion test at some point in time. Therefore, as a *very rough guesstimate,* on the average for U.S. cases, $P(B_1$; virion test) ≈ 107,800/330,000,000 = 0.000327 = 0.0327% (ca. 7/23/20). Note that the CDC website set "new infec-

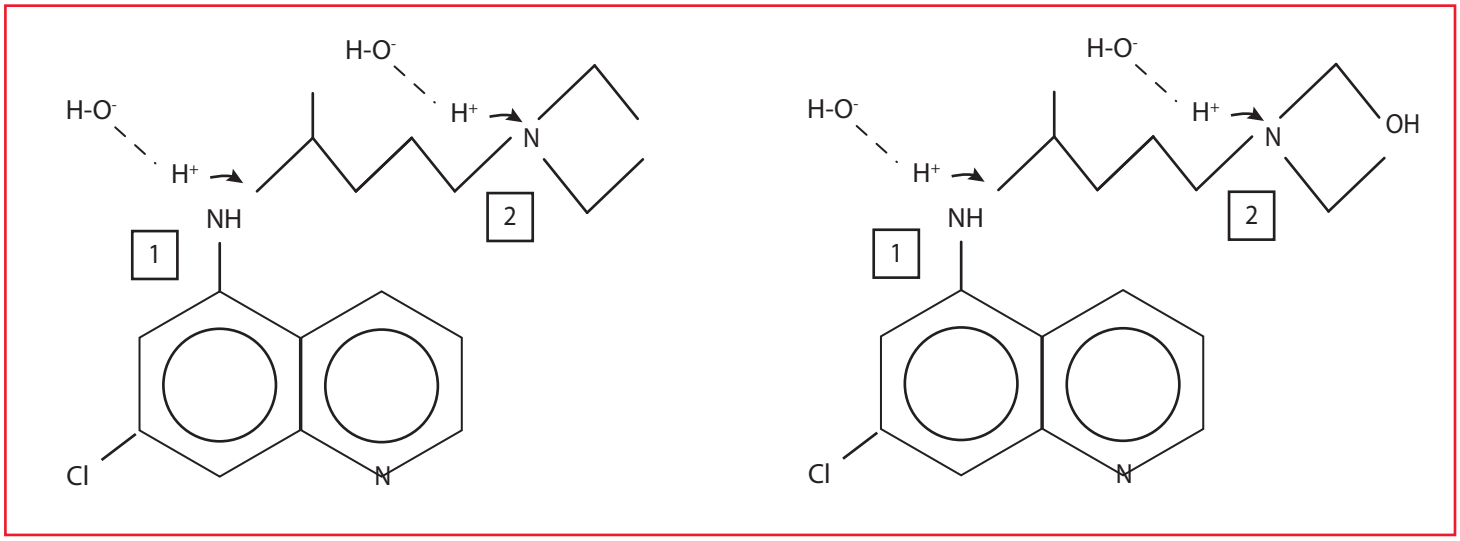

Figure 5 — The molecular structure of chloroquine (left) and hydroxychloroquine (right; note the presence of the hydroxyl group, hence the name). It is the ability of nitrogen to bind a proton (hydronium ion) captured from a water molecule that makes these two species weak bases. In general, if "B" represents chloroquine or hydroxychloroquine, and if H^+ represents a hydronium ion, then the acid base reaction is of the form $H^+ + B \leftrightarrow HB^+$, where HB^+ is called a conjugate acid. *Each of the molecules above has two sites (nitrogen atoms) upon which a water molecule can make an electrophilic attack, labeled "1" and "2." Site "1" is called a* secondary amine *site, and site "2" is called a* tertiary amine *site. Both contribute to the basicity of these two drugs. Normally, the secondary amine site ("1") would be most important. However, in this case, since aryl rings (benzene rings) are attached to site "1" its basicity is reduced. Consequently, the conjugate acid dissociation constants for the two sites are close at 7 and 9.2. These acid/base properties are just what is required to neutralize the interior acidity of a liposome (to be discussed, also see Figures 2 and 6) — not so neutral as to require toxic amounts of the drug but not so basic as to disrupt the membrane of the all-important liposome. An understanding of the acid/base properties of chloroquine and hydroxychloroquine is essential for understanding the action of these drugs on COVID-19-infected cells.*

tions" on July 23 at 70,160, in rough agreement with new infections estimated from deaths. For false negatives, it is desirable for $P(\mathbf{B_1} \mid \mathbf{A})$, the probability that a person has COVID-19 *given* that their test for the disease was negative, to be as small as possible. A recent report from Johns Hopkins University has found that virion tests may yield false negatives more than 20% of the time (i.e., $P(\mathbf{A} \mid \mathbf{B_1}) \geq 0.2$), while $P(\mathbf{A} \mid \mathbf{B_2})$ was not specified [Ref. 13]. If $P(\mathbf{A} \mid \mathbf{B_2})$ is used as an independent variable, the graph in Figure 4 emerges for $P(\mathbf{B_1} \mid A)$, the probability (for the U.S. population with 0.000327% *actively* infected) that a person who tests negative actually has COVID-19.

This graph demands some explanation. Note that the curve (lower boundary of shaded region) does not approach zero as the accuracy of the test approaches 100% in a ***healthy isolated population*** (no false positives). That's because in the U.S. population as a whole there are ***infected people*** for whom a false negative is possible. On the other end of the $P(\mathbf{A} \mid \mathbf{B_2})$ scale the curve rises as one might expect for a less accurate test. Why are the values of $P(\mathbf{B_1} \mid \mathbf{A})$, false negatives (vertical axis), so small? It is because *active* COVID-19 (i.e., shedding virions) is very rare when $P(\mathbf{B_1}) = 0.000327$. Therefore, *on the (lower) curve* of Figure 4, a 0.01% false negative rate for the whole U.S. population can be achieved with only about 65% accuracy in an isolated healthy population! Bayes' Theorem can yield some surprising and useful results. However, 65% is a moving goal for manufacturers. As infection spreads through the population, higher demands are made on the accuracy of tests if a 0.01% false negative rate is to be maintained in the general population. If $P(\mathbf{B_1}) = 0.0004$ (a modest increase), then tests must be *at least* 80% accurate in an isolated healthy population. It is fortunate that there is just barely enough information to make these calculations.

It is unfortunate that $P(\mathbf{A} \mid \mathbf{B_2})$ (equal to one minus the fraction of false positive reactions) was not measured for the pristine population on the island of Guam (pop. 165,000), for as many manufacturer's tests as were available, before March 1, 2020. On March 15, the first COVID-19 case was detected on Guam [Ref. 14]. Tests employing nasal swabs with the normal *in-vivo* (live subject) mix of mucosal microbial flora, or blood having the normal mix of interfering proteins, are more realistic than tests employing *in-vitro* (test tube) microbes or proteins.

However, they are also slower and more expensive. The Pacific islands really are miniature "worlds apart," to use a phrase coined by the eminent biologist David Attenborough. The opportunity to test a more or less genetically homogeneous population with a similar diet and environment may have been lost on Guam, but perhaps another isolated population can be found for the accurate determination of $P(\mathbf{A} \mid \mathbf{B}_2)$. Tragically, the coronavirus was detected in the Northern Mariana Islands (pop. 53,883) on March 28, 2020. The Earth is running out of uninfected places.

In summary, each tested person (and their attending physician) should be aware of the false positive/negative frequency of their particular manufacturer's test, and this should be compared to the local frequency of occurrence of clinical disease before making any decisions based on virion or antibody COVID-19 tests. There are many published statistics concerning the number of positive coronavirus tests. But what tests? Were they virion or antibody tests? Who manufactured those tests, and what do these manufacturers claim for $P(\mathbf{A} \mid \mathbf{B}_1)$ and $P(\mathbf{A} \mid \mathbf{B}_2)$ in the false positive and false negative cases? *Because of these uncertainties, deaths and new hospital admissions are probably the clearest way of determining how the coronavirus infection is progressing in the population*.

Can a Vaccine Be Made?

This is a difficult question because the author, in a sense, must try to see into the future, which is always uncertain. As of this writing, there are over 100 programs underway to develop a vaccine for the coronavirus [Ref. 15]. That doesn't necessarily mean that a vaccine is "just around the corner." There are several practical difficulties to overcome. The first difficulty has already been discussed. Namely, the high mutation rate of RNA virus genomes in general. If a vaccine is developed against envelope glycoproteins today, will it convey an immunity that is any good six months from now (see the previous discussion on antigenic drift)? Will booster shots be needed? How many and how often?

Another practical problem concerns the cultivation of human coronaviruses. The virus is difficult to grow in cultured cells. Consequently, mouse hepatitis virus has been used as a *model* for the coronavirus genus, and much of what is known about the molecular biology of coronavirus replication comes from these models. The use of models is common among biochemists. For example, when studying plant cells, the tobacco plant is often used as a model specifically because it is so easy to cultivate. However, when preparing a vaccine, a model organism is usually not good enough. Medicine got lucky with smallpox (variola). The model organism, so to speak (cow pox virus, *vaccinia virus*), was readily available from infected cows so that cultivation was not a problem. Cow pox was also relatively safe if it infected humans (low virulence), and similar enough to smallpox that it was able to convey immunity (the first vaccine). In fact, the word *vaccination* comes from the Latin *vaca* for "cow."

Traditionally, vaccines are prepared by two methods. The first involves killing a disease-causing microbe with a chemical agent like formalin (e.g., Salk vaccine), or by the use of heat or radiation. The resulting microbial fragments can be used as the foreign agents (*antigens*) which, when injected into a subject, excite the human immune response. The second method involves preparing an *attenuated strain* that is safe for use in a live vaccine (e.g., polio Sabin vaccine). Attenuated strains can be produced by manipulating genes to eliminate virulence factors. Both of these traditional approaches require the difficult-to-produce coronavirus stock as a starting point.

Today, genetically modified organisms (GMOs) can be created that are capable of expressing a protein that is a *clone* of, say, a glycoprotein like S in the coronavirus envelope. When this foreign protein (or even part of such a protein) is injected into a subject it excites the immune response. Although a yeast may seem like an unlikely host for such a protein, that is exactly what can be done! Furthermore, yeast can be cultivated by the ton in enormous fermentation vessels of 100,000- to 150,000-liter capacity filled with liquid growth medium so that copious amounts of vaccine can be produced. It's a modern extension of an ancient technology originally intended for the production of alcoholic beverages.

On the plus side, COVID-19 is due to a single type of virus. The common cold is due to more than 200 types of (rapidly evolving) rhinoviruses and coronavirus [Ref. 3], so preparation of a vaccine is impractical for colds. Fortunately, the spectrum of viruses involved in COVID-19 is much narrower. There have also been reports that people who have recovered from COVID-19 can get "the same" infection again. Is the reinfection really "the same," or is it just another strain (minor variation) of the usual virus? Or, has the patient's immunity simply expired? Or, much more frightening, is COVID-19 one of those few diseases (like gonorrhea [Ref. 3]) that does not excite, or can evade, the human immune response, in which case you can get the same infection over and over again and a vaccine cannot be prepared? This latter scenario does not seem to be the case at this time.

So a few technical difficulties must be overcome. However, in the author's opinion, a vaccine can probably be prepared. The principal questions are how long will

it take, will it be safe, how effective will it be, how long will immunity last, and how many shots will be needed to establish immunity? Currently (June 2020), the U.S. is beginning final phase tests on the first vaccines. These tests will involve 30,000 subjects [Ref. 15]. But, even if these tests fail, many more will follow. Brazil will also be conducting large scale tests using a Chinese vaccine.

What About Post-Infection Pharmaceuticals?

It needs to be stated unequivocally, up front, that there are ***no FDA-approved*** *treatments as of this writing. While there are some promising results from trials underway,*

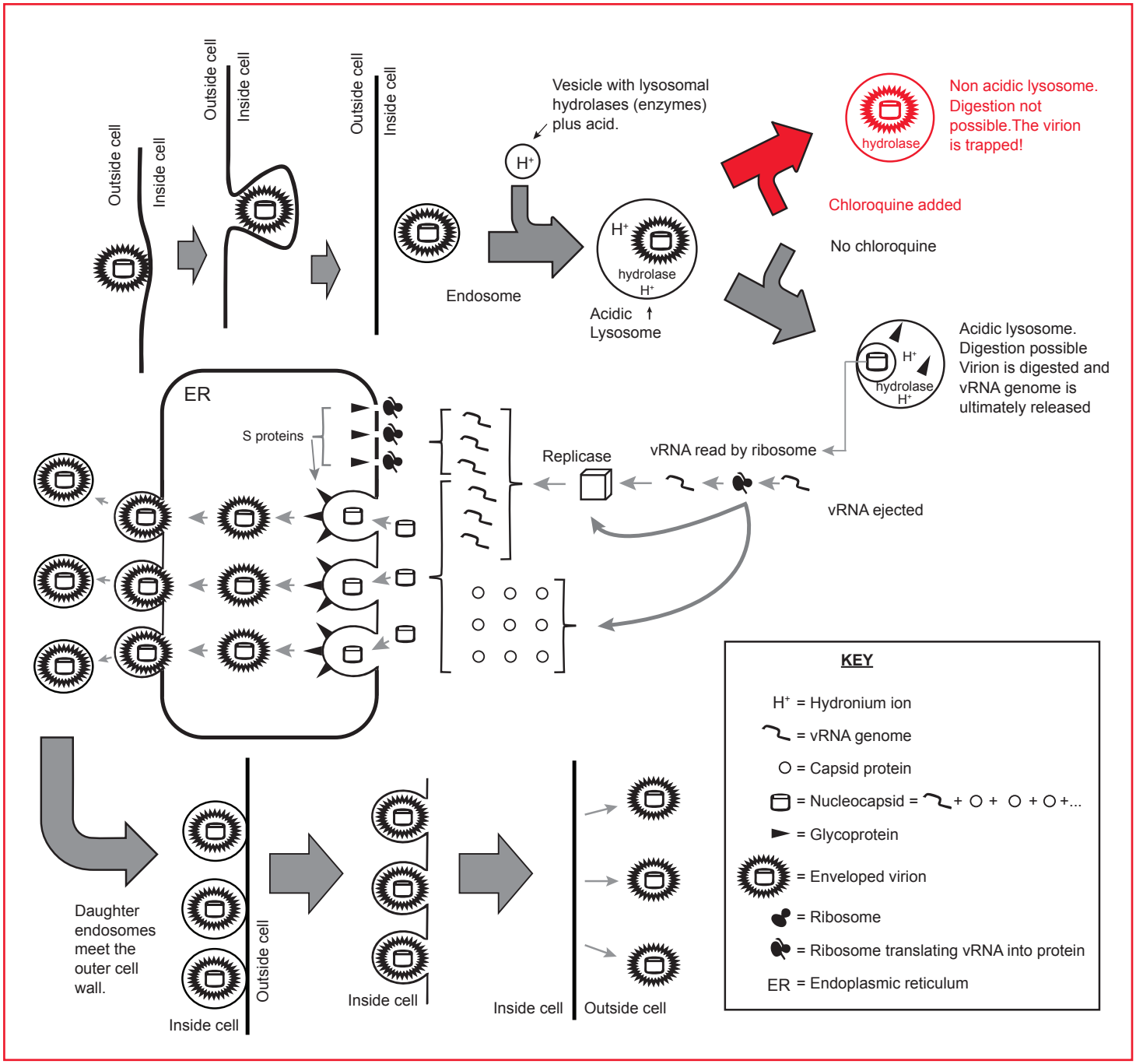

Figure 6 — The life cycle of the coronavirus from infection of a host cell to ejection of its daughter virions (gray path [Refs. 19-21]). This schematic should not be taken too literally. The details are complex, and many steps have been omitted. Significant rearrangement of the host cell's membranes is involved during infection and the process is still not completely understood. This cartoon is primarily intended to summarize the topology of lipid bilayers encountered by a coronavirus (which can be confusing) and to indicate the point in the life cycle that is exploited by chloroquine and hydroxychloroquine (red path). This life cycle may seem unnecessarily complicated until one realizes that the only membrane in a cell that can add sugar ("glyco") to a protein, to make the all-important glycoprotein S, is that of the endoplasmic reticulum (ER). Therefore, daughter nucleocapsids must bud through this membrane.

and clinicians may be treating patients with various remedies because they have nothing else, any use of these treatments for COVID-19 patients is considered experimental. Further complicating matters is the fact that use of some of these drugs has become "political" [Ref. 16]. The discussion here will begin with a description of the two controversial drugs chloroquine and hydroxychloroquine; their history, their uses, their bioactivity, and their side effects. Next in-vitro (in-glass or test tube) experiments will be discussed, followed by a discussion of drug safety and in-vivo (in-life or clinical) trials. Finally, this section will conclude with a discussion of dexamethasone.

First of all, C*hloroquine* (also known as *Aralen*) is a *synthetic alkaloid* (C18H26ClN3; see Figure 5) that was discovered in 1934 by Hans Andersag and coworkers at Bayer laboratories and was further developed during WW II (German pat. 683,692 [1939], U.S. pat. 2,233,970 [1941]) and has become the main weapon against human malaria, being much less toxic than its predecessor quinine [Ref. 17]. Therefore, this drug has a long history and has been well tested. Chloroquine, and its congener hydroxychloroquine (Figure 5) having even fewer side effects, display high oral bioactivity and are used to treat several illnesses in addition to malaria (amebic liver abscesses, lupus, rheumatoid arthritis, and other autoimmune disorders). When taken, just over 50% of chloroquine is bound to plasma proteins. Chloroquine also binds to DNA, and is a weak base. Furthermore, it is extensively concentrated several hundredfold in tissues. In addition, its principal metabolic product may also be pharmacologically active. These properties may be important factors in the drug's bioactivity, and several mechanisms have been proposed to explain its action against malaria [Ref. 11]. Chloroquine is generally well tolerated. At lower prophylactic doses (5 mg base / kg of body mass) few side effects can be observed in most people [Ref. 17]. In fact, the author has taken chloroquine many years ago during his travels through the tropics without apparent consequence. At therapeutic doses used for acute malarial attacks (25 mg base / kg), gastrointestinal upset may occur as well as transient headaches and visual disturbances [Ref. 17]. Chloroquine is safe for use at normal dosages during pregnancy, and its use certainly outweighs the danger of malaria to the mother [Ref. 17]. At high doses, hypotension (low blood pressure), lowered myocardial function, vasodilation, and abnormal electrocardiograms may result [Ref. 17]. These are the facts as they are known.

In-vitro tests have shown promising results, but even these have their skeptics. One official of the Biomedical Advanced Research and Development Authority (BARDA) said that there was "not a lot of enthusiasm based on just vitro data" [Ref. 16]. On the other hand, Stanford University researchers, in an article describing the mechanism of chloroquine's action, note that the drug (a base that can accumulate in tissues) can pass through a cell's membranes and increase the interior pH (decrease the interior acidity) of *liposomes*, where invading virions are trapped (see Figure 6). When that happens, the life cycle of the coronavirus is disrupted and mature infectious daughter virions cannot be produced (see Figure 6). As they state, "And here's where chloroquine comes in to defend a foundering cell. ... That's what happens in a dish (i.e., a petri dish), anyway" [Ref. 18]. They further state that "watching how they (i.e., chloroquine and hydroxychloroquine) work in a lab dish can teach researchers a lot. Insights and tweaks from their observations could yield similar — but more effective — drugs with their inadequacies ironed out and their SARS-CoV-2 (i.e., COVID-19 virion) fighting strengths honed to a sharp edge." [Ref. 18]. Findings of this kind have moved chloroquine and hydroxychloroquine into clinical trials. Finally, the reader should keep in mind the caveat stated by the Stanford research team concerning the connection between in vitro and in vivo testing [Ref. 18]:

> "First the cells in a dish aren't exactly the same as the cells in living tissues affected by SARS -CoV-2. Second, the environment surrounding, say, a lung cell in a living person's body is quite different from the one in a culture dish. And third, there's this thing called side effects. You don't see those in a dish."

When trying to perfect a new drug, safety is always high on the list of concerns. The U.S. Food and Drug Administration (FDA) issued the following warning (italics added): "FDA cautions against use of hydroxychloroquine, or chloroquine for COVID-19 *outside of the hospital setting or a clinical trial* due to risk of heart rhythm problems." [Ref. 22]. This warning seems prudent for an old drug used in a new way (called *repurposing*), especially in light of the well-known abnormal electrocardiograms at high doses. *If* these drugs do provide a patient with some measure of relief then, as with any drug, the risks that they pose (possibly minor or rare in a given case) may be tolerable. Another safety charge was that "Bayer's chloroquine was not safe" [Ref 16] after the pharmaceutical giant donated 3 million tablets to the federal stockpile "from a factory in Pakistan that had not been certified by the F.D.A. as safe." [Ref. 16]. It's

a curious charge leveled against the company that had originally discovered the drug. In any case, it would seem simple enough to use a mass spectrometer to assay its purity. Other types of tests can be done as well, whatever the FDA deems necessary.

Finally, the results of in-vivo experiments have been justifiably controversial. Clinical trials of chloroquine and hydroxychloroquine have yielded mixed results [Refs. 16, 18, 23-28]. It's puzzling, but the discrepancies will have to be resolved! The molecular structure of these drugs may have to be adjusted.

Clinical trials are very difficult because people, unlike the model tobacco plants discussed in the previous section, are not genetically uniform and are not grown under identical conditions in a test tube or on a flat. A person's life history, genetic predispositions, time at which they were infected, the level of infection, whether they were previously treated with other drugs, and hundreds of other variables that may be difficult to determine, all come into play.

It is hoped that large enough trials will average out some of these factors, but then there is the issue of sample size. Basically, the only way to be absolutely certain that a sample size is large enough is to repeat a trial with an even larger population. If the statistics remain the same, you're done! If not, an even larger experiment needs to be made. It's a daunting task, and slow. The efficacy issue has been further clouded by the resent *retraction* of a paper in *The Lancet* where researchers claimed they did "not observe any benefit of hydroxychloroquine or chloroquine" [Ref. 29]. Much more serious is the behavior of some Brazilian researchers "accused of poisoning their patients with high doses of chloroquine to give the drug (i.e., chloroquine) a bad name" [Ref. 30]. These incidents say nothing about the efficacy of the drugs being tested. However, they do indicate that caution should be used when evaluating the claims of a single researcher or team. There are more than 50 trials of hydroxychloroquine underway in the U.S. Hopefully, these trials will sort out the truth by producing a consensus of opinion.

Another post-infection therapeutic agent recently announced is the steroid dexamethasone [Ref. 31]. This drug down-regulates the body's overwhelming autoimmune response to the coronavirus's attack on cells lining a patient's airways and lungs. Perhaps chloroquine and its congener, also used to treat autoimmune disorders as mentioned previously, do something similar. As with chloroquine, dexamethasone is a well-known drug that is readily available in most pharmacies. But, also like chloroquine, its effectiveness for COVID-19 patients in controlled scientific experiments is yet to be established. Could "up to 5,000 deaths … have been prevented" in the U.K. if dexamethasone had been used from the beginning of the pandemic to treat the sickest patients, or "Do we just cause prolonged suffering in one in eight patients on vents?" by using this drug [Ref. 32]. The scientific community will have to wait for clinical trials to be completed with published results in peer reviewed journals before the efficacy of post-infection pharmaceuticals is fully known and understood. In the meantime, physicians will try to do what they can to alleviate symptoms on an emergency basis.

"Home remedies often seem harmless, but they have downstream effects that are difficult to predict. This author is concerned that masses of people, finding fraudulent information on the internet, may glean a certain comfort from that, causing them to be less careful about masking, social distancing, handwashing and other measures that our public health officials are trying to promote."

There are literally hundreds of therapeutic agents being tested. These include antivirals used to treat Middle Eastern Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) because the general virion genomic layout and replication kinetics are the same as for COVID-19. Still other drugs exploit traditional choke points such as interference with coronavirus replicase (see Figure 6). Although cellular pH control and using anti-inflammatory agents are only two possible coronavirus therapies, the three drugs discussed here are the ones that have grabbed the headlines recently. It can only be hoped that some very useful compounds will be discovered by the current round of tests. The author truly wishes that he had more definite answers to the questions about the value of post-infection therapies but, at this point, it's a waiting game. Unfortunately, *time* is what the human race does not have!

What About Home Remedies?

Home remedies often seem harmless, but they have downstream effects that are difficult to predict. This author is concerned that masses of people, finding fraudulent information on the internet, may glean a certain comfort from that, causing them to be less

careful about masking, social distancing, handwashing and other measures that our public health officials are trying to promote. With that being said, two very common remedies come to mind.

After years of controversy following Linus Pauling's famous announcement, it was definitely established that taking mega-doses of vitamin C at the onset of a cold can be beneficial [Ref. 3]. Whether that translates into an improved ability to fight COVID-19 depends on the validity of using the 200 or so assorted rhinoviruses and coronaviruses responsible for the common cold as models for COVID-19. The connection seems weak.

The next common remedy is zinc. It seems that "zinc lozenges" (containing a zinc compound) taken at the first sign of a sore throat helps retard the onset of cold symptoms [Ref. 3]. It is notable that zinc is used in the human body by a large number of DNA-binding proteins. The protein's binding site is called a *zinc finger* [Ref. 1]. Perhaps these proteins are important in the cold-fighting process. Will the ingestion of certain zinc compounds improve resistance to COVID-19? No one knows, but it is under investigation.

Conclusion

If nothing else, the author hopes that this discussion on our current pandemic has gathered together in one place most, or at least many, of the fragments of information scattered throughout the media and the literature, while at the same time discarding many fragments of misinformation. Furthermore, it is hoped that many of the common questions in people's minds concerning the current pandemic have been answered as objectively, scientifically, understandably and completely as possible in a single brief article.

However, in the end, information is only useful to those who understand it. And, once again, the author finds himself in a position that is critical of the safety personnel within many companies and organizations. Many of the employees, and most of the managers, are "paper" engineers or administrators who have forgotten most of their education, and never had the laboratory experience or breadth of training in biochemistry, microbiology, physics, mathematics and medicine to effectively design a safe work environment. The coming of the cold and flu season this fall and winter may have dire consequences for public health and economics unless a vaccine, or some other intervention, can soon be perfected, manufactured in bulk, and distributed. However, that seems quite unlikely. It must always be remembered that the world's pharmaceutical companies will have to manufacture enough materials for an overpopulated world of 7.7 billion people. These are sobering thoughts for dark times!

Stay well!

About the Author

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