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Analyses of the 2020 Nobel Prize in physiology or medicine: taking the hepatitis C virus from mystery killer to preventable and treatable disease

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Analyses of the 2020 Nobel Prize in Physiology or Medicine: Taking the Hepatitis C Virus from Mystery Killer to Preventable and Treatable Disease

Harvey J. Alter, Michael Houghton, and Charles M. Rice share the 2020 Nobel in Physiology or Medicine for their contributions to the identification, treatment, and eventual elimination of the hepatitis C virus (HCV). The then novel molecular techniques they used have laid the groundwork for future work on HCV and other viruses. This article will give a brief overview of the hepatitis disease and the work of this year's laureates. It will also take a bibliometric look at the scientist's research and add to the discussions about the way modern science is done and the limitations of prestigious science awards being awarded to only three awardees.

Keywords: hepatitis C virus, HCV, Harvey J. Alter, Michael Houghton, Charles M. Rice, Nobel

Introduction

During a time when the world is paying attention to the global impact of the SARS-CoV-2 virus, work that was done half a century ago on another deadly virus has come into the spotlight. On October 5, 2020 the Nobel Assembly at the Karolinska Institutet awarded the 2020 Nobel in Physiology or Medicine to researchers for their work on the hepatitis C virus. As SARS-CoV-2 has made utterly apparent to the world, there are many steps that go into the eventual screening, testing, and treatment of a viral disease. This year's three laureates each played a pivotal role in taking the hepatitis C virus from an unknown killer to a detectable and treatable disease. Their discoveries have led to improved screening of blood products, greatly decreasing a major transmission route of the virus, the development of antiviral drugs, and hopefully eventually a vaccine (Nobelprize.org 2020a).

There have been at least three previously awarded Nobels that are related to the work of this year's award. In 1912 Dr. Alexis Carrel was awarded the prize for "his work on vascular suture and the transplantation of blood vessels and organs" (Nobelprize.org 2020c). Carrel's work transformed the way blood transfusions were performed, greatly increasing their safety and efficacy which eventually led to them becoming a medical norm. Dr. Karl Landsteiner won the award in 1930, thirty years after his discovery of the ABO blood groups. Once his work was recognized and utilized it led to a drastic decrease in transfusion rejections (Nobelprize.org 2020d; Starr 1998). Most directly related to this year's award is the 1976 Nobel provided to Dr. Baruch Blumberg for his work on the hepatitis B virus(NobelPrize.org 2020e) One of this year's laureates, Dr. Alter, worked with Dr. Blumberg on some of his work that led to the identification of, and tests for, the hepatitis B virus antigen. This year's award adds to these previous

discoveries and perfectly fits the model of the Nobel in Physiology or Medicine in that it takes decades to realize the full impact of certain discoveries. The honor is awarded to researchers for specific discoveries, not lifelong work. These discoveries mark a point where the 'science of before' is replaced by a 'science of after.' Before the discoveries of this year's laureates, the hepatitis C virus was unknown and its disease was considered an ongoing plague (Nobelprize.org 2020a). After these discoveries, the virus is nearly eliminated from the donor blood supply and currently has a 95% cure rate (World Health Organization 2020b).

Another feature of the Nobel in Physiology or Medicine is that it tends to be awarded to three scientists, the maximum allowed number of awardees, who all made a significant contribution towards the awarded topic. This norm of three awardees is likely due to the incredible amount of work from individuals and groups that is the basis of a lot of modern science. Previously, it was not uncommon for individuals to make discoveries substantial enough to warrant the award. In fact, the first thirty-two awards (from 1901-1933) went mostly to single awardees, with only five being divided between two individuals. By the mid 1940s, the maximum of three awardees began to take place and since the mid-sixties this has become the norm, closely followed by pairs. In the past sixty years, there have only been eight years with the award going to a single laureate (NobelPrize.org). While this trend of awarding the prize to the maximum number of three seems to fit the trend of science being done by many individuals or groups, it has also become a source of contention. The number of people who contribute to groundbreaking research, including that of this year's award, greatly exceeds three and most of the individuals who work on the research are left without the acknowledgement (Ledford 2020). This is an issue faced by many prestigious science awards but no resolution seems to be in sight (Houghton 2013).

The Science

A brief history of blood transfusions

In 1818 Dr. James Blundell performed the first successful human transfusion, saving a woman who was hemorrhaging (The American Red Cross). Over the next eleven years, Blundell attempted nine additional transfusions with a total of five patients surviving. Between 1847 and 1856, Dr. Alfred Higginson transfused seven patients and even though five died he proceeded to affirm the usefulness of the procedure. In 1908 Dr. Alexi Carrel performed a direct transfusion using a new technique by connecting a father to his five-day old daughter to save her life. Soldiers of the American Civil War were transfused with mixed success. All of these transfusions were done with little or no concern about where the blood came from and even after Dr. Landsteiner identified ABO blood types in 1900, testing of blood did not occur for decades. (Dr. Carrel was aware of Landsteiner's work and while he didn't test for ABO, or anything else, it did influence his decision to use the father as the donor.) The benefit of using blood during war

was seen again in WWI and by WWII blood and blood products were considered essential. After the wars, surgeries and other medical treatments became more advanced and elaborate, resulting in a growing need for blood beyond trauma victims. Products derived from blood were also being used and seen to expand and improve the lives of those with chronic diseases such as hemophilia. The 1970s saw a blood products boom as well as an increase in transfusion-acquired hepatitis, leading to tens of thousands of deaths (Starr 1998).

In between the two world wars, in 1925, medical professionals in London established the world's first municipal donor panel, the Greater London Red Cross Blood Transfusion Service. By this time, they understood enough to screen their donors with a physical exam and by testing for syphilis. They also tested for ABO blood type. Americans were behind the British in this regard, not creating the American Red Cross until WWII and not testing for anything until finally screening for syphilis beginning in 1947. Besides the lack of testing, another major issue facing the blood business was that in order to keep up with demand, blood banks often relied on commercial blood, referring to it as professional blood since many donors sold their blood as a source of income. It was also common practice to have blood draws at prisons. Paid donors and prison donors were later shown to have increased rates of hepatitis and other blood transmitted diseases. The huge demand for plasma and plasma-derived products also added complications, as plasma is pooled from multiple donors, greatly increasing the risk of transfusion-acquired diseases. Eventually these products ended up being safer than whole blood since most of them could be heat-treated, but this was only done after the connection was undeniable (Starr 1998).

According to the American Red Cross, someone in the U.S. needs a blood product every two seconds. These lifesaving products include whole blood, plasma, packed red blood cells, platelets, or plasma derived proteins. Fortunately, one single donation can be used to save up to three lives; unfortunately less than 38% of the population is actually eligible to donate these products (The American Red Cross). Today, not only does the U.S. get its blood from volunteer donors that undergo an extensive screening process, the list of tests done to screen the donated blood for infectious agents is extensive. Viruses (including hepatitis B and C, HIV, human T-cell lymphotropic virus type 1, West Nile, Zika, and cytomegalovirus), bacteria (including Treponema pallidum and Trypanosoma cruzi, as well as a general bacterial screening), and the parasite Babesia are all tested for in multiple ways to ensure safe blood transfusions (U.S. Food and Drug Administration 2020). The hepatitis C virus is on this list and able to be tested and screened for due to the work of this year's Physiology or Medicine Nobel Laureates.

Hepatitis

Hepatitis, or inflammation of the liver, can present in an acute or chronic state. Chronic hepatitis can lead to liver cirrhosis, failure, and cancer (Gupta 2018). While the primary cause of Hepatitis is viral, specifically from Hepatitis B or C viruses, other causes include toxins, drugs including alcohol, or other diseases. Hepatitis A, D, and E viruses, and cytomegalovirus, are also viral

causes of Hepatitis (Selzner and Levy 2020). Hepatitis A virus (HAV) is an RNA virus (Picornaviridae family) transmissible via contaminated food and water resulting in an acute disease that, although occasionally deadly, is usually relatively mild and leads to immunity from further infections (Gupta 2018). Hepatitis B virus (HBV) is a DNA virus (Hepadnaviridae) that is transmissible via bodily fluids including from mother to child and blood transfusions. HBV can result in both acute or chronic disease states and prior to the 1980s was the sole leading cause of Hepatitis (Selzner and Levy 2020; Gupta 2018). Hepatitis C virus (HCV) is a genetically variable RNA virus (Flaviviridae family) that is transmitted via blood products and also results in both acute and chronic conditions. While some patients can clear the virus during the acute stage, the majority will end up in the chronic state and, due to the presentation of the disease, will often not know they are sick until significant liver damage has already occured (Gupta 2018; Newton 2013). Hepatitis D virus is a single species RNA virus in the *Deltavirus* genus that is transmissible sexually and via blood products but only causes significant disease when co-infected with HBV. Hepatitis E virus is also a single species RNA virus but in the *Hepevirus* genus and is transmissible via contaminated food and water similarly to HAV (Gupta 2018).

Hepatitis affected humans long before the identification of the viruses. Descriptions of hepatitis symptoms date as far back as ancient Sumeria. Early Romans and Greeks, including Hippocrates, noted hepatitis symptoms including jaundice and even realized the transmissible nature of the disease. By the late 19th century, the realization that the disease was transmitted via injections or bodily fluids began taking shape when an epidemic at a shipyard seemed to be tied to smallpox vaccinations; only those who got the vaccination showed symptoms of hepatitis. Other vaccinations and medical treatments involving injections over the years also seemed to lead to outbreaks of the disease, including an outbreak in the U.S. Navy in 1942 where over 50,000 individuals caught hepatitis from a contaminated yellow fever vaccination. These, and other, outbreaks provided data that led to the belief that there were two types of transmissible hepatitis (Trepo 2014). These two types were initially classified as infectious hepatitis (IH) or serum hepatitis (SH) based on their transmission routes and incubation times (Blumberg, Sutnick, and London 1969). As transfusions and blood products were used with increasing frequency in wars, including the American Civil War, the Franco-Prussian War, and both World Wars, evidence supporting the idea of serum hepatitis and establishing a link between the disease and blood transfusions grew. Confirming this link was difficult to do though. There was only one well documented direct link of a blood transfusion seeming to cause hepatitis during WWII, and no documented links prior to that. Eventually the link was established, but there was no way to prevent it besides asking donors to not donate if they had previously had the disease. It wasn't until after the link was undeniable that a post-WWII surplus of plasma donated to the American Red Cross was shown to be contaminated with the disease (Starr 1998).

In the 1960s Blumberg identified the hepatitis B antigen (HBsAg), which was originally called the Australia antigen, leading the way for the testing of blood donors. With this discovery, it was also noted that the incidence of the antigen was found in one out of every 1000 Americans (about 2% higher in drug users), many of whom were asymptomatic and thus would not have previously been screened out of blood donations (Blumberg, Sutnick, and London 1969; NobelPrize.org 2020e). Blumberg's work led to the development of a HBV test that the U.S. Food and Drug Administration mandated for use in 1972, although some blood banks had started using the test the previous year. Although this test was only 15% effective it resulted in a 25% decrease in the risk of transfusion-acquired HBV; a later test mandated in 1975 was 40% effective (Starr 1998; Hepatitis B Foundation). A task force in the early 1970s estimated that there were 17,000 transfusion-acquired hepatitis cases and 850 deaths a year. However, this estimate was much lower than the CDC's estimate of 3,500 deaths (Starr 1998). It was around this time that Harvey Alter was making his landmark discovery that, together with Blumberg's work, would eventually start to turn everything around.

The work of the laureates

Non-A, non-B

The work leading to this year's Nobel in Physiology or Medicine begins in the late 1990s with Dr. Harvey J. Alter. Alter, who worked with Blumberg on his Nobel winning hepatitis B work, performed methodical studies showing that there was another blood transmissible agent in addition to HBV that was leading to transfusion-acquired hepatitis (Nobelprize.org 2020a). Alter's (1972) article initially discussing this discovery was built on previous studies that looked at the infection rates of HBV from commercial blood compared with volunteer blood. In this prospective study, Alter looked at open-heart surgery patients, and the blood they were transfused with, using first bi-weekly then monthly blood tests to look for any sign of hepatitis transmission. The findings from this and previous studies showed that removing all hepatitis-B antigen (HBAg) positive blood from the donor pool would result in a 25% reduced transmission of hepatitis. However, if commercial blood was removed and only non-tested voluntary blood used, there would be a reduction of 70%. If only HBAg negative voluntary blood were to be used, the hepatitis transmission rate would drop by 85%. While these important results regarding HBAg and blood screening were the original goal of the study, the study also demonstrated that there was likely another blood-borne cause of hepatitis.

All told, nine of the 126 patients (7.1%) ended up having hepatitis, as indicated by a certain increase in their serum glutamic pyruvic transaminase (SGPT). These patients contracted the disease although they were transfused with blood that was thoroughly tested for HBAg by four different methods - agar gel diffusion, counterelectrophoresis, complement fixation, and hemagglutination. Three of these patients had SGPT results indicating hepatitis after a short incubation period while still testing negative for both HBAg and its antibody, anti-HBAg. Five

patients had indicative test results only after a long incubation period and they ended up testing positive for HBAg. One patient, patient J.K., fell into both groups first having a short incubation with no HBAg or anti-HBAg detected and then again having indicative test results after a longer incubation, followed by testing positive for HBAg. All four patients who were negative for HBAg during the short incubation infection were thoroughly tested to eliminate as many false negatives as possible. Counterelectrophoresis, complement fixation, hemagglutination inhibition, and solid-phase radioimmunoassay were all run with negative results. Additionally, samples from eighty of the eighty-two units of blood donated to these four patients were retested with solid-phase radioimmunoassay, all with negative results. All of this testing strongly suggested that the four patients who experienced short incubation periods became ill from a non-HBV cause. J.K.'s experience of having both distinct infections further strengthened this finding leading to Alter's declaration that the patient's "posttransfusion hepatitis was the result of at least two different viruses." All four of these patients also tested negative for cytomegalovirus, another potential cause of hepatitis. In fact, Alter and his team took great care to eliminate other possible infectious agents not from blood transfusions and other possible causes of increased SGPT (Alter et al. 1972).

That six patients (including J.K.) ended up positive for HBAg even after thorough screening of donated blood indicated that the current screening methods for HBAg in donated blood were insufficient. Solid-phase radioimmunoassay on donated serum was shown to be a possible way of improving blood screening (Alter et al. 1972). A similar study on 108 patients published a few years later supported these findings. From these 108 patients, there were twelve cases of hepatitis despite thorough screening of blood for HBAg with conventional methods. Of the twelve cases, eight were classified as non-B hepatitis. The incubation period for this non-B type (mean of 9.4 weeks) fell in between that of HAV and HBV and seemed to be less severe during the acute phase but could lead to serious chronic conditions. This non-B agent, inferred to be a virus, also proved to not be serologically related to HAV, cytomegalovirus, or Ebstein-Barr virus (Alter et al. 1975). Ultimately, Alter concluded that if all transfused blood came from volunteers and was tested for HBAg using solid-phase radioimmunoassay then the "vast majority" of any transfusion-acquired hepatitis would be due to a non-A, non-B hepatitis virus (or viruses).

Following these studies indicating a non-A, non-B hepatitis virus, Alter did a small study investigating the possible transmissibility and infectiousness of this unknown virus. At the time of this study, 60-90% of transfusion-acquired hepatitis was not tied to either HAV or HBV, strongly suggesting that non-A, non-B hepatitis agent was leading to many transfusion-acquired hepatitis cases. To explore this, Alter's team took plasma or serum from patients in both acute and chronic phases of non-A, non-B hepatitis, as well as from a blood-donor seemingly responsible for two cases of transfusion-acquired hepatitis. These samples were then injected into otherwise healthy chimpanzees who all had subsequent blood tests and liver biopsies

indicating hepatitis. These results add to previous evidence that while non-A, non-B hepatitis results in nearly identical liver cell damage as HAV and HBV, it is caused by a separate agent with a distinct incubation time from either HAV or HBV. Critically, non-A, non-B also was found to have a positive correlation between infection and contaminated blood. Notably, this correlation is evident with blood from both acute and chronic patients, indicating that not only is non-A, non-B a transmissible agent, it results in a chronic carrier state (Alter et al. 1978).

Identification by cloning

Now that there was strong evidence of a transmissible non-A, non-B hepatitis virus, the next step was to identify it. This turned out to be a difficult task as researchers spent the decade following Alter's work unsuccessfully attempting to identify the mysterious virus. In the late 1980s, Michael Houghton and his team began working on the problem assuming that the underlying issue was that the level of non-A, non-B antigen present in samples was too low for current techniques to pick up. They worked around this issue by creating a cDNA library from non-A, non-B infected chimpanzee plasma (Choo et al. 1989). A DNA library is a collection of DNA fragments from biological specimens of interest. These fragments are stored, usually in a bacterium or yeast, with specific DNA 'handles' allowing them to be easily replicated and stored. The 'c' in cDNA refers to complementary DNA, and cDNA libraries are created from DNA that is reverse transcribed from the source RNA (Tomso 2003). Houghton's cDNA library was run against serum from a known chronic non-A, non-B hepatitis patient and ultimately a cDNA clone, clone 5-1-1, was identified. The homologous RNA to this cDNA clone was shown to be single stranded and between 5,000 to 10,000 nucleotides in size. Other characteristics of the RNA were also identified. Specifically, it was determined that the immunoreactive polypeptide encoded in the cDNA has a singular open reading frame (ORF, or protein-coding region) that reacts with the non-A, non-B patient's serum. Following these promising initial results, serum from eleven other non-A, non-B hepatitis patients and ten healthy donors were tested against the 5-1-1 clone. Seven out of the eleven non-A, non-B serums had similar results, while none of the healthy donor serums reacted. These results indicated that, despite a decade of inability to detect a viral antigen or antibodies, at least one cause of non-A, non-B hepatitis was an RNA virus. It is in this seminal article that the hepatitis C virus is given its name. Additionally, Houghton was able to determine that HCV most likely belongs to either the togaviridae or flaviviridae families (it was later shown to belong to flaviviridae) (Choo et al. 1989). This work was the first time these molecular techniques had been used in such a way and not only did it result in identifying HCV, it provided a technique for the future identification of other unknown agents (Nobelprize.org 2020a).

In the same issue of *Science* that published the HCV naming article, Houghton, Alter, and others published another article further indicating that HCV was a major cause of non-A, non-B transfusion-acquired hepatitis. This study was designed to capture the long missing HCV antibodies. In the study, the researchers created a polypeptide with the recently identified ORF

and a human enzyme. This polypeptide, classified as C100-3, was then expressed in yeast. Yeast with C100-3 were then used to line plates which attracted and bound HCV antibodies. The bound antibodies also bonded to a radioactive second antibody used for detection. Using this method, the researchers tested blood from known non-A, non-B infected chimpanzees, as well as positive blood donors and the recipients of the donated blood (Kuo et al. 1989). Specificity and sensitivity were thoroughly tested using a samples panel Alter had previously created specifically for non-A, non-B assays. This panel had come to be the ultimate test for identifying the non-A, non-B agent and nineteen other promising candidates had failed it (Alter 2014). When Houghton's candidate was run against the panel, the results showed that the method his lab developed was a valid way of identifying HCV antibodies and was a method that could be used to test blood donations as a way to further decrease the number of transfusion-acquired hepatitis cases (Kuo et al. 1989).

HCV replication and infectious nature

While Houghton was successful in identifying a RNA clone of HCV, work towards finding a treatment was lagging and nearly a decade after Houghton's work, over 1% of the world population suffered from chronic HCV. Research had identified various properties of HCV that many hoped could lead to more information about the virus' infectious nature, improving its replication in-vitro, and ultimately a treatment, but progress was slow (Kolykhalov et al. 1997). Charles M. Rice and his team tackled this problem by looking at the molecular requirements of HCV replication. They were able to identify a specific region in the virus' genome important to replication. Rice also found areas of the genome that seemed to actually disrupt replication and was eventually able to engineer a version of the virus that did not have these hindering sequences. This engineered virus was then shown to cause hepatitis when injected into chimpanzees, demonstrating that HCV could indeed lead to transfusion acquired hepatitis on its own (NobelPrize.org 2020e).

Rice took serum from a patient known to be in the early stages of the disease to create a combinatorial library resulting in 233 prescreened clones. (Combinatorial libraries are basically collections of compounds or molecules resulting from a type of chemistry called combinatorial chemistry.) Thirty-four of these prescreened clones were initially tested and resulted in RNA that did not lead to infection in chimpanzees. This failed experiment led Rice's group to further investigate by sequencing some of the clones. This sequencing is what identified the areas of the HCV genome that disrupts infectivity. The sequencing also pointed out the areas in the genome that had a lot of variability (specifically the ends and the section coding for a glycoprotein of the viral envelope). Using this information, Rice's team created ten different clones with identifying markers and injected them into two HCV negative chimpanzees. Serum samples and biopsies from both chimpanzees showed they had contracted HCV and the researchers were able to track the clones by the identifying markers. In addition to showing that HCV can replicate and is a causative agent of hepatitis, this work provided a model and detailed information about the HCV

RNA allowing for further study of the virus. It was now possible to produce as much infectious RNA as needed for genetic analysis leading to information "on virus evolution, pathogenesis, and host immune response" (Kolykhalov et al. 1997).

Significance and current/future science

In the early 1970s the rate of non-A, non-B transfusion-acquired hepatitis was estimated to be twenty cases per 1000 units of blood. With multiple units being used per transfusion these numbers meant that 33% of transfusions would result in non-A, non-B hepatitis (Alter et al. 1972). By 1989, studies indicated that up to 10% of all transfusions resulted in hepatitis and that more than 90% of transfusion-acquired hepatitis in the U.S. was non-A, non-B (Kuo et al. 1989). Despite the drop in hepatitis cases (likely as a result of restricting commercial blood and blood from prisoners, as well as increased donor screening due to the AIDS epidemic) a screening test for non-A, non-B was desperately needed (Kuo et al. 1989; Starr 1998). A decade after Alter's determination of a non-A, non-B agent, Houghton was able to identify the agent as the hepatitis C virus and provide a method for the crucial screening test which began being used in blood banks in the early 1990s. Nearly another decade later, Rice definitively confirmed that HCV could replicate and cause the disease, while setting the stage for further research into the virus, including treatment. The three winners of this year's Physiology or Medicine Nobel provided the information needed to identify and screen for a chronic and deadly disease. Since their work, even more has been done to improve screening tests, improving the safety of blood transfusions and products, and to provide treatment to patients.

Comparing modern blood banking and transfusion to that of even fifty years ago can be quite a shock. The beginning was very much trial and error, especially as the science of transfusion was being explored. In the book *Blood: an epic history of medicine and commerce* Dr. Bertram M. Bernheim, a distinguished surgeon at the Johns Hopkins Hospital, is quoted as saying "in looking backwards I marvel at our recklessness." While volunteer donors were used, a large supply of blood, especially that used for pooling plasma, came from paid donors. Paying donors attracted those that were at higher risk of disease including intravenous drug users. They also did no preliminary tests for diseases, or even for blood type. Today, only voluntary blood is used for patients, all donors are heavily screened, and all donated blood and products are tested with an array of tests. Whereas HCV infected an estimated 180,000 transfusion recipients in 1984, the identification of HCV, and HBV earlier, has led to the ability to test and screen for the viruses, resulting in an almost complete elimination of transfusion-acquired hepatitis (Starr 1998; Oldstone 2020, 198). Of course, if blood products are not properly screened before transmission, both viruses can still be transmitted. Other transmission routes for HCV include injectable drug use, inadequate sterilization of medical instruments including needles, sexual encounters that result in exposure to blood, and, rarely, mother to child during birth (World Health Organization 2020b). The near elimination of transfusion-acquired hepatitis has done wonders, and the work from all the laureates has led to additional work finding treatments and even cures for HCV. All

of this raises the possibility that, with a lot of international collaboration, HCV may be eliminated (NobelPrize.org 2020e).

According to the World Health Organization, 325 million people currently live with a Hepatitis infection and it is estimated that 71 million of these have chronic HCV. In 2016, almost 400,000 people globally died from HCV complications (World Health Organization 2020b, 2020a). The U.S. Centers for Disease Control and Prevention (CDC) estimates that as of 2018 there were 96,800 viral hepatitis cases in the U.S. with HCV causing an estimated 50,300 infections. For the year of 2018 alone, there were 137,713 new cases of chronic HCV reported and 214 new cases of perinatal HCV infection. This same year saw a documented 17,533 deaths from a hepatitis viral infection, with HCV being the listed cause of death for 15,713 of those (Centers for Disease Control and Prevention 2020). As is evident by these numbers, and noted by this year's Nobel press release, blood-borne hepatitis, and specifically HCV, is a global public health concern on par with HIV and tuberculosis (NobelPrize.org 2020e). The 2017 *World Health Organization (WHO) Global Hepatitis Report* prominently states that in 2016 "the World Health Assembly endorsed the *Global Health Sector Strategy (GHSS) on viral hepatitis 2016–2021*. The GHSS calls for the elimination of viral hepatitis as a public health threat by 2030 (reducing new infections by 90% and mortality by 65%)" (World Health Organization 2017). In their report, the WHO notes that the primary innovations needed include more affordable and available HCV testing and treatment, and an HCV vaccine which to date has not been successfully created. When looking at 2018 data from death certificates in fifty states and D.C. that shows 3.72 deaths per 100,000, it can seem that this goal of eradicating HCV is unlikely. However, this number is nearly 26% lower than the mortality rate in 2014 (Centers for Disease Control and Prevention 2020). Progress is being made.

Treatment for HCV once had limited success, resulting in more toxicity related side-effects than actual elimination of the virus. Today, treatment usually consists of direct-acting antiviral (DAA) treatments which result in minimal side-effects and a cure rate of over 95% (Holmes, Rutledge, and Chung 2019). DAAs are named such because they target HCV directly by acting against specific proteins created in the HCV life cycle. The specific proteins with current corresponding DDAs are NS3/4A protease, NS5B RNA-dependent RNA polymerase, and NS5A protein. There are four main types of DDAs - three that work against the three specific proteins, and one group that is made up of agents that work against a combination of the proteins. These drugs prevent their specific protein, or proteins, from functioning, thus stopping the HCV life cycle (HepatitisC.net 2017). These drugs have made a vital impact on HCV and the WHO currently recommends all patients over the age of 12 with a chronic infection to use them, specifically those that affect multiple proteins (World Health Organization 2020b). These drugs, and all the screening tests and previous treatments, would not be possible without the work of this year's three laureates. Their work will continue to be built upon as current and future researchers work towards a vaccine.

The Scientists

Harvey J. Alter

Life, Education, and career

Harvey J. Alter was born to Jewish parents in 1935 at Beth Israel Hospital in Manhattan, NY (NobelPrize.org 2020e; Alter 2014). Alter's father, a businessman with an interest in medicine, greatly influenced him and he planned to go into medicine from a young age. The exact area of interest in medicine changed over time from ophthalmology to pediatrics and eventually to internal medicine with a focus on hematology. He attended the University of Rochester in Rochester, NY for his undergraduate and medical degrees (Alter 2014). He received his BA in 1956 and his MD in 1960; he did his medical internship at Strong Memorial Hospital, also in Rochester (Seeff and Ghany 2014). Interestingly, his application for admittance to the medical school included being interviewed by George Hoyt Whipple, a previous Nobel in Physiology or Medicine laureate. In 1961, he started a position at the National Institutes of Health in the Division of Biological Standards (DBS) which allowed him to avoid the draft for war he was scheduled to report to a few days later. This series of events was one of the most pivotal events in his life in that if he had been drafted he likely would have spent his life in private practice, not in research. While at the DBS, Alter worked on febrile transfusion reactions. This work led to a collaboration with Baruch Blumberg on work that eventually led to the discovery of the Australia/HBV antigen. Alter notes that the discovery of the Australia antigen set the course for his career, and it was a discovery that "wasn't even remotely on [their] radar." Beyond the chance to work on such groundbreaking research, Alter credits Blumberg with teaching him a great deal that helped him in his career, including perseverance and the value of saving samples (Alter 2014).

In 1964, Alter went to the University Hospitals in Seattle for a brief second-year residency and then to Georgetown University for a hematology fellowship under Charles Rath. Alter stayed at Georgetown, becoming Director of Hematology Research until 1969 when the NIH invited him back to continue working on transfusion-associated hepatitis (Alter 2014; Seeff and Ghany 2014). He returned to the Clinical Center's Department of Transfusion Medicine as a senior investigator (NobelPrize.org 2020e). He has since become the Clinical Center's Chief of the Infectious Diseases Section and Associate Director of Research in the Department of Transfusion Medicine (NIH Clinical Center 2020). He is also the W. Thomas London Distinguished Scientific Advisor to the Hepatitis B Foundation and an Honorific Professor of the Baruch S. Blumberg Institute (Hepatitis B Foundation 2020).

Not surprisingly, Alter has an incredibly long list of awards and honors. A much abbreviated list includes 1977 U.S. Public Health Service Distinguished Service Medal; 1992

American Association of Blood Banks (AABB) Karl Landsteiner Award; 1994 British Blood Transfusion Service James Blundell Prize; 2000 Hepatitis B Foundation's Distinguished Scientist Award; 2002 International Society of Blood Transfusion Presidential Award and American Liver Foundation Distinguished Scientist Award; 2004 American College of Physicians Award for Outstanding Work in Science and Institut National de la Santé Et de la Recherche Médicale (French equivalent of the NIH) International Award for Science (Alter was the first to receive this award); 2006 Society for Advancement of Blood Management Presidential Award; and 2011 AABB Tibor Greenwalt Memorial Award and Lectureship and American Association for the Study of Liver Diseases' Distinguished Achievement Award (NIH Clinical Center 2020). The award from the American Association for the Study of Liver Diseases is one of the awards Alter most "cherishes" (Alter 2014). As is usual for Physiology or Medicine Nobel awardees, Alter also received the Albert Lasker Clinical Research Award in 2000 (shared with Houghton) and the Canada Gairdner International Award in 2013 (Canada Gairdner Foundation 2013). In 2015 he was awarded the Centers for Disease Control and Prevention's Fries Prize for improving health, the Grand Hamdan International Award, and the University of Rochester's Charles Force Hutchison and Marjorie Smith Hutchison Medal (Sheikh Hamdan Bin Rashid Al Maktoum Award for Medical Sciences). In addition to the awards he has received over the years, Alter also has been honored with distinguished positions including being appointed as NIH Distinguished Investigator in 2008 and elected into the American Association of Physicians in 1992, both the Institute of Medicine and the National Academy of Sciences in 2002, and to Mastership in the American College of Physicians in 2003 (NIH Clinical Center 2020). He also received the University of Rochester's Distinguished Alumnus Award leading to him giving the Whipple Lecture (named for the Nobel Laureate who interviewed him for medical school) in the same auditorium where he gave Grand Rounds as a student (Alter 2014).

During his time at Georgetown, in 1965, Alter married Barbara Bailey. They were married twelve years and had two children, Mark an M.D./Ph.D. and Stacey a teacher, and four grandchildren. In 1984 Alter met his current wife Diane. The second marriage grew his family by two stepdaughters, Lydia and Erinn. Alter's work continues to be based on his HCV work where he utilizes the samples he has stored over the years to study the evolution of the virus' natural history (Alter 2014).

Bibliometrics

Not surprisingly, Alter's career has resulted in numerous impactful publications. The Web of Science (WoS) Core Collection was searched on November 9, 2020 to gather bibliometric data on Alter. It needs to be noted that this collection only goes back to 1965. While this time frame covers the majority of Alter's publishing career, including the article he considers his "first publication in a high-impact journal," a few of his earliest articles are not included in the data (Alter 2014). (The article he is referring to is "Further Studies on a New Human Isoprecipitin System (Australia Antigen)" published with Blumberg in *Blood* in 1966. This article has been

cited 117 times as of November 2020 according to WoS, and 190 times according to Google Scholar.) According to the WoS Core Collection, Alter has 504 articles from 1965 - 2020. He has published at least three articles a year since 1971, publishing at least ten articles a year in twenty-six different years. His most prolific years were 1991 and 2001 when he published twenty-two articles each year (see Figure 1).

[Figure 1 - Alter publications per year]

These publications have resulted in 37,691 citations (36,684 without self-citations) averaging almost 75 citations per article. These citations come from 22,969 citing articles (22,704 without self-citations). Even though his publishing career didn't start much before 1965, he has had multiple citations each year since then and every year after 1970 has had over 100 citations a year, with eighteen years having over 1,000 (see Figure 2). One of his earliest articles, "A New Antigen in Leukemia Sera," covering his work with Blumberg that identified the Australia Antigen is also one of his most highly cited articles. His top two most highly cited articles are two articles co-authored with Houghton and three of his other top five are on his HCV work (see Table 1). He has an H-index of 95.

[Figure 2 - Alter citations per year]

[Table 1 - Alter top five cited articles]

The journals Alter publishes in the most are all high-impact journals. The journal *Blood* which Alter mentions as his first high-impact journal, although he first published in it prior to journal metrics, is his seventh most frequently published in journal. He has published seventeen additional articles in the journal since his first in 1966. All of his top five journals have at least twenty of his articles, with *Hepatology* publishing eighty-eight over his career (see Table 2).

[Table 2 - Alter top journals published in]

Michael Houghton

Life, Education, and career

Michael Houghton was born in 1949 in London, England (Semeniuk 2020). Houghton became interested in the medical sciences when he was seventeen after becoming inspired when reading about Louis Pasteur (Qaiser 2020). After secondary school, Houghton received a scholarship to attend the University of East Anglia where he studied biology and earned his BS in 1972 (Seeff and Ghany 2014; News Archive 2019). After East Anglia, he attended King's College in London

where he studied biochemistry and worked on identifying the human beta interferon gene (Seeff and Ghany 2014). He earned his PhD in biochemistry from King's in 1977. In the early 1980s, Houghton emigrated to the U.S. where he worked at G.D. Searle & Company (a pharmaceutics company) and then moved to the Chiron Corporation where he did his Nobel winning work (NobelPrize.org 2020e).

After twenty-five years at Chiron, Houghton went to Epiphany Biosciences in 2007 to be their Chief Scientific Officer. A few years later he moved to Alberta Canada to work at the University of Alberta where he was invited as one of the first awardees of the Canada Excellence Research Chair (CERC), an award for "outstanding national and international scientists" given by the Canadian government (Seeff and Ghany 2014; Semeniuk 2020). He is still at University of Alberta as a Professor in the Department of Medical Microbiology & Immunology and the Li Ka Shing Professor of Virology (Qaiser 2020; NobelPrize.org 2020e).

In addition to the CERC, Houghton has received many awards. These awards include the Karl Landsteiner Memorial Award and Lectureship (1992), Robert Koch Medal (1993), William Beaumont Prize in Gastroenterology (1994), Clinical Lasker Award (2000), Dale A. Smith Memorial Award (2005), William H. Prusoff Hep Dart Lifetime Achievement Award (2009), and Canadian CLF-CASL gold medal (2012) (American Gastroenterological Association ; AABB ; Li Ka Shing Institute of Virology ; Virology Education). Houghton was also awarded the 2013 Canada Gairdner International Award with Alter. However, Houghton declined the award when the number of awardees was not extended to include his colleagues Qui-Lim Choo and George Kuo who worked closely with him on his HCV work (Qaiser 2020). In addition to these awards, the University of East Anglia awarded Houghton an honorary Doctorate of Science in 2019 (News Archive 2019).

Houghton continues to work on HCV and is currently working on an HCV vaccine which his lab started working on in 2012. They currently have one that is in late pre-clinical stage testing. Vaccines are not new to Houghton as he developed one for SARS-CoV-1 in 2004 and is currently working on one for SARS-CoV-2 (COVID-19) (University of Alberta 2020).

Bibliometrics

According to data pulled from the Web of Science Core Collection on November 9, 2020, Houghton started publishing in 1974 and has published 345 articles since. Similar to Alter, Houghton has published every year since his first publication, publishing at least ten articles a year in fourteen different years. His most prolific year was 1991 when he published thirty-eight articles (see Figure 3). His publications have been cited 41,549 times since 1975 (40,400 times without self-citations) from 20,130 different articles (19,934 without self-citations). Houghton's articles have received at least 100 citations a year for thirty-three of the past forty-seven years.

Seventeen of those years have seen at least 1000 citations a year with 1992 having 2439 citations (see Figure 4).

[Figure 3 - Houghton publications per year]

[Figure 4 Houghton citations per year]

Houghton's two most highly cited articles are the two published back-to-back in *Science* where he shares his Nobel-winning work (the second of which is Alter's most highly cited article). His most highly cited article "Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome" has been cited 5715 times in the Web of Science Core Collection as of November 9, 2020, averaging over 178 citations a year (see Table 3). As is usual with science Laureates, Houghton tends to publish in high impact journals. His most highly published in journal is *Hepatology* where he published fifty-three (15.36%) of his articles (see Table 4). An interesting note about his fifth most published in journal, *Lancet*, is that it was named after the tool originally used for bloodletting in the early days of blood transfusions (Starr 1998).

[Table 3 - Houghton top five cited articles]

[Table 4 Houghton top journals published in]

Charles M. Rice

Life, Education, and career

Charles M. Rice was born in Sacramento, California in 1952 to an insurance claims adjuster father and stay-at-home mom. Rice attended the University of California, Davis (UC Davis) where he earned a B.S. in zoology in 1974. While at UC Davis, Rice took a biology course taught by Dennis Barrett that greatly influenced him. At Barrett's suggestion, Rice attended a summer course at the Marine Biological Laboratory at Woods Hole, Massachusetts which further guided him towards being a basic scientist rather than his initial plan of being a veterinarian. After receiving his BS, Rice became a teaching assistant for the Woods Hole course and then, in 1975, returned to California to attend the California Institute of Technology where he studied biochemistry. During his graduate studies, Rice did research on the Sindbis virus while working in James Struass' virology lab. After receiving his PhD in 1981, Rice continued on in Struass' lab to do his postdoctoral work on the yellow fever virus (Nair 2011). In 1986, Rice moved on to the Washington University School of Medicine in St. Louis, Missouri where he established his own research group and became a full professor in 1995 (NobelPrize.org 2020e). Fifteen years

later in 2001, a virologist Rice greatly admired, Stephen Goff, recommended him for a position at Rockefeller University as Scientific and Executive Director of the Center for the Study of Hepatitis C. This center was the first hepatitis C research center in the northeastern U.S. and combined experts from Rockefeller, New York Presbyterian Hospital, and Cornell University's Weill Medical College (Nair 2011). Rice took the position and has remained at Rockefeller where he is currently a faculty member in the David Rockefeller Graduate Program, and the Tri-Institutional M.D.-Ph.D. Program, as well as being the Maurice R. and Corinne P. Greenberg Professor in Virology (Rockefeller University).

Rice has also received numerous awards and honors. The same year he moved to St. Louis to start his own lab, 1986, he won the Pew Charitable Trust scholarship for young biomedical researchers (Nair 2011). He also has been awarded the M.W. Beijerinck Virology Prize (2007), Rockefeller University Distinguished Teaching Award (2010), Robert Koch Award (2015), InBev Baillet Latour Health Prize (2016), Lasker-DeBakey Clinical Medical Research Award (2016), and Memorial Sloan Kettering Cancer Center C. Chester Stock Award (2017). Additionally, he has been elected into the National Academy of Sciences and the American Association for the Advancement of Science Fellowship (Rockefeller University). He has also been a member of the American Society for Microbiology since 1983 and was an editor of the Journal of Virology from 2001-2008 (ASM Communications 2020).

Rice continues working on developing in vitro culture and animal models to study virus replication and innate immune responses at Rockefeller (Rockefeller University).

Bibliometrics

Rice's first publication, according to data pulled from the Web of Science Core Collection on November 9, 2020, was in 1984. Since then he has published at least two articles a year for a total of 509 articles. Twenty-five years have seen at least ten articles a year published, with both 2013 and 2014 having twenty-nine articles (see Figure 5). All of these publications have resulted in 55,309 citations (52,916 without self-citations) from 28,389 different articles (27,974 without self-citations). These citations started the year he first published and he has had over 100 citations a year since 1989 with every year since 2002 seeing over 1,000 citations a year. The year with the most citations is 2015 with 3,683 (see Figure 6). Rice's h-Index is 125.

[Figure 5 - Rice publications per year]

[Figure 6 - Rice citations per year]

While Rice has worked and published with both Houghton and Alter, none of his top five most cited articles are co-authored with either of his fellow laureates. Two of his most highly cited articles, "Complete replication of hepatitis C virus in cell culture" and "Efficient initiation

of HCV RNA replication in cell culture," are on HCV (see Table 5). Surprisingly, the article cited by the Nobel Prize press release as the article describing his Nobel winning work, "Transmission of hepatitis C by intrahepatic inoculation with transcribed RNA," is not in the list of his top five, at least not yet. Rice publishes in similar journals to Houghton and Alter. The journal *Virology* is his most published in journal with 128 (25.20%) of his 509 articles (see Table 6).

[Table 5 - Rice top five cited articles]

[Table 6 - Rice top journals published in]

Laureates thoughts on the Nobel

In Alter's (2014) *Hepatology* Master's Perspective, he talks about the many points in his life that lead to him doing the research he did. In particular, he mentions his decision not to go with Blumberg when the latter left the NIH, somewhat lamenting that if he had he may have shared the Nobel with Blumberg in 1976. The way the Nobel for Physiology or Medicine works though, it most likely ended up better for him that he did not go with Blumberg. Obviously, Alter now has a Nobel for the work he possibly wouldn't have done had he gone with Blumberg, but it is also likely he would not have shared the 1976 with Blumberg even if he did stay with him. The Physiology or Medicine Nobel has not adapted as medical research has advanced. Nobel-worthy research is almost always performed by groups of researchers, both working together in the same lab and working off of each other, or independently, in different labs. Alter himself lists numerous individuals he has worked with and depended on saying "My composite of awards leaves me with pangs of guilt that I Have been singled out for accomplishments that were achieved only through vital collaborations" (Alter 2014). The Nobels however are awarded to the Principal Investigators of the labs determined to have made the most impact. Due to this, the Nobel awarded to Blumberg most likely would not have been shared with Alter. Similarly, those whose work greatly impacted HCV research are not included in this year's award.

Houghton has also experienced this dichotomy between how science is done and the most prestigious awards for science. When he was awarded both the Robert Koch Prize in 1993 with Daniel Bradley and the Clinical Lasker Award in 2000 with Alter he lamented that his colleagues Choo and Kuo did not share the award. As mentioned previously, the same concerns were raised in 2013 when he, Alter, and Bradley were awarded the 2013 Canada Gairdner International Award. He declined the Gairdner after failing to convince the Gairdner Foundation to add Choo and Kuo to the list of recipients. While he had long expressed his frustrations with this rule of three awardees, declining the Gairdner, and its $100,000 prize, was a way to more visibly express his frustrations and stay true to his beliefs (Houghton 2013). Since then he has been asked about his thoughts on refusing the Nobel. His answer has been that refusing the Nobel, while something he considered, doesn't make as much sense primarily because the Gairdner has

made exceptions to the rule of three before while the Nobel never has. He also mentions that this is tied to Alfred Nobel's will which led to the creation of the Nobel Prizes (Qaiser 2020). Nobel's will actually reads as if all the awards, not just Physiology or Medicine, should be given to a single awardee. Each line dictating the five Nobel areas begins with "one part to the person." The full line for the Physiology or Medicine prize reads "one part to the person who made the most important discovery within the domain of physiology or medicine" (Nobelprize.org). Houghton's sentiment is accurate though as rule of three comes from the conclusion of the legal battle between the Nobel Foundation (the foundation created to manage the awards) and Alfred Nobel's heirs which states "that the amount of a prize thus awarded shall under no circumstances be less than sixty percent of that portion of the annual yield of the fund that shall be available for the prize award, nor shall it be divided into more than three prizes at most" (Nobelprize.org).

During his Nobel interview with Thomas Perlmann, Rice also discusses the many researchers who had contributed to the HCV work. He expresses surprise that he was chosen as the third recipient and sees himself more as a representative of all those who did not get the acknowledgement (Nobelprize.org 2020b). This issue of researchers being overlooked due to the rule of three is likely going to continue to come up. Science has changed since the provisions were set in 1898. Those provisions are set though and how and if they can be changed will likely be up to lawyers, if the Nobel Foundation even sees fit to try to change them.

Conclusion

This year's Nobel in Physiology or Medicine has been awarded to three scientists for their work over the past thirty years leading to the identification, screening, and treatment of the hepatitis C virus (HCV). Their work builds on each other's and has provided information and techniques for future investigations into HCV, and other viruses. Harvey J. Alter received his third of the award for his work that showed the existence of a non-A, non-B cause of transfusion-acquired hepatitis. Michael Houghton identified the mystery agent as being a virus and named it the hepatitis C virus. Charles M. Rice then identified the area of the HCV genome responsible for replication and confirmed that HCV could act as a sole agent causing hepatitis. These discoveries have taken the world from dealing with an unknown agent causing 33% of blood transfusions to result in hepatitis, to a known and treatable virus and near zero cases of hepatitis caused by blood transfusions. A vaccine is also now on the horizon as is the global elimination of the virus.

References

AABB. "List of Past AABB Award Recipients." Accessed November 16, 2020. http://www.aabb.org/about/awards/Pages/recipientspast.aspx.

Alter, Harvey J. 2014. "The road not taken or how I learned to love the liver: A personal perspective on hepatitis history." *Hepatology* 59 (1): 4-12.

https://doi.org/https://doi.org/10.1002/hep.26787. https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.26787.
Alter, Harvey J., Paul V. Holland, Andrew G. Morrow, Robert H. Purcell, Stephen M. Feinstone, and Yasuo Moritsugu. 1975. "Clinical and serological analysis of transfusion-associated hepatitis." *The Lancet* 306 (7940): 838-841.
Alter, Harvey J., Paul V. Holland, Robert H. Purcell, Jerrold J. Lander, Stephen M. Feinstone, Andrew G. Morrow, and Paul J. Schmidt. 1972. "Posttransfusion hepatitis after exclusion of commercial and hepatitis-B antigen-positive donors." *Annals of internal medicine* 77 (5): 691-699.
Alter, Harvey J., Paul V. Holland, Robert H. Purcell, and Hans Popper. 1978. "Transmissible agent in non-A, non-B hepatitis." *The Lancet* 311 (8062): 459-463.
American Gastroenterological Association. "William Beaumont Prize in Gastroenterology." Accessed November 16, 2020. https://gastro.org/membership/recognition-awards/william-beaumont-prize-in-gastroenterology/.
ASM Communications. 2020. Nobel Prize Awarded to Power Trio of ASM Contributors. Accessed November 16, 2020.
Blumberg, Baruch S., Alton I. Sutnick, and W. Thomas London. 1969. "Australia antigen and hepatitis." *JAMA* 207 (10): 1895-1896. https://jamanetwork.com/journals/jama/article-abstract/344257.
Canada Gairdner Foundation. 2013. "Harvey J. Alter MD." Accessed November 12, 2020. https://gairdner.org/award_winners/harvey-j-alter/.
Centers for Disease Control and Prevention. 2020. Viral Hepatitis Surveillance — United States, 2018. edited by U.S. Department of Health and Human Services. Atlanta, GA.
Choo, Qui-Lim, George Kuo, Amy J Weiner, Lacy R Overby, Daniel W Bradley, and Michael Houghton. 1989. "Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome." *Science* 244 (4902): 359-362. https://science.sciencemag.org/content/244/4902/359.long.
Gupta, Satya P. 2018. "Viral Hepatitis." In *Studies on Hepatitis Viruses*, 1-14.
Hepatitis B Foundation. "Hepatitis B Vaccine History." Accessed October 29, 2020. https://www.hepb.org/prevention-and-diagnosis/vaccination/history-of-hepatitis-b-vaccine/#:~:text=The%20hepatitis%20B%20virus%20was,of%20an%20American%20hemophilia%20patient.
---. 2020. "Nobel Prize to Hepatitis B Foundation and Blumberg Institute Advisors." Accessed November 12, 2020. https://www.hepb.org/news-and-events/news-2/nobel-prize-to-hepatitis-b-foundation-and-blumberg-institute-advisors/.
HepatitisC.net. 2017. What Are Direct-Acting Antivirals for HCV Treatment? Accessed October 23, 2020.
Holmes, Jacinta A., Stephanie M. Rutledge, and Raymond T. Chung. 2019. "Direct-acting antiviral treatment for hepatitis C." *The Lancet* 393 (10179): 1392-1394. https://doi.org/10.1016/S0140-6736(18)32326-2.
Houghton, Michael. 2013. "Three isn't the magic number." *Nature Medicine* 19 (7): 807-807. https://doi.org/10.1038/nm0713-807.
Kolykhalov, Alexander A., Eugene V. Agapov, Keril J. Blight, Kathleen Mihalik, Stephen M. Feinstone, and Charles M. Rice. 1997. "Transmission of hepatitis C by intrahepatic

inoculation with transcribed RNA." *Science* 277 (5325): 570-574. https://science.sciencemag.org/content/277/5325/570.long.

Kuo, George, Qui-Lim Choo, Harvey J. Alter, Gary L. Gitnick, Allan G. Redeker, Robert H. Purcell, Tatsuo Miyamura, Jules L. Dienstag, Miriam J. Alter, Cladd E. Stevens, Gary E. Tegtmeier, Ferruccio Bonino, M. Colombo, William S. Lee, C. Kuo, Kavita Berger, Jeffrey R. Shuster, Lacy R. Overby, Daniel W. Bradley, and Michael Houghton. 1989. "An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis." *Science* 244 (4902): 362-364. https://science.sciencemag.org/content/244/4902/362.long.

Ledford, Heidi. 2020. "The unsung heroes of the Nobel-winning hepatitis C discovery." *Nature* 586 (7830): 485. https://www.nature.com/articles/d41586-020-02932-y.

Li Ka Shing Institute of Virology. "Director." University of Alberta. Accessed November 16, 2020. https://www.ualberta.ca/li-ka-shing-institute-virology/li-ka-shing-applied-virology-institute/director.html.

Nair, Prashant. 2011. "Profile of Charles M. Rice." *Proceedings of the National Academy of Sciences* 108 (21): 8541-8543. https://doi.org/10.1073/pnas.1105050108. https://www.pnas.org/content/pnas/108/21/8541.full.pdf.

News Archive. 2019. "UEA Announces Honorary Graduates for 2019." University of East Anglia Accessed November 16, 2020. https://www.uea.ac.uk/news/-/article/uea-announces-honorary-graduates-for-2019.

Newton, David E. 2013. "Hepatitis." In *The Gale Encyclopedia of Public Health*, edited by Laurie J. Fundukian, 442-450. Detroit, MI: Gale.

NIH Clinical Center. 2020. "Harvey Alter, MD." Meet Our Doctors. Department of Health and Human Services. Accessed November 11. https://clinicalcenter.nih.gov/meet-our-doctors/halter.html.

NobelPrize.org. "All Nobel Prizes in Physiology or Medicine." Nobel Media AB 2020. Accessed November 6, 2020. https://www.nobelprize.org/prizes/lists/all-nobel-laureates-in-physiology-or-medicine/.

---. "Full text of Alfred Nobel's will." Nobel Media AB 2020. Accessed November 17, 2020. https://www.nobelprize.org/alfred-nobel/full-text-of-alfred-nobels-will-2/.

---. "Statutes of the Nobel Foundation." Nobel Media AB 2020. Accessed November 18, 2020. https://www.nobelprize.org/about/statutes-of-the-nobel-foundation/#par4.

---. 2020a. "Announcement of the 2020 Nobel Prize in Physiology or Medicine." Nobel Media AB 2020. Accessed October 5, 2020. https://www.nobelprize.org/prizes/medicine/2020/prize-announcement/.

---. 2020b. "Charles M. Rice--Interview." Nobel Media AB 2020. Accessed November 18, 2020. https://www.nobelprize.org/prizes/medicine/2020/rice/interview/.

---. 2020c. "The Nobel Prize in Physiology or Medicine 1912." Nobel Media AB 2020. https://www.nobelprize.org/prizes/medicine/1912/summary/.

---. 2020d. "The Nobel Prize in Physiology or Medicine 1930." Nobel Media AB 2020. https://www.nobelprize.org/prizes/medicine/1930/summary/.

---, October 5, 2020, 2020e, "Press release: The Nobel Prize in Physiology or Medicine 2020," https://www.nobelprize.org/prizes/medicine/2020/press-release/.

Oldstone, Michael B. A. 2020. *Viruses, Plagues, and History: Past, Present, and Future*. New York: Oxford University Press.

Qaiser, Farah 2020. Virologist Michael Houghton Is One Of The 2020 Nobel Prize In Medicine Recipients. *Forbes*.

Rockefeller University. "Charles M. Rice, Ph.D.". Heads of Laboratories. Accessed November 16, 2020. https://www.rockefeller.edu/our-scientists/heads-of-laboratories/893-charles-m-rice/.

Seeff, Leonard B., and Marc G. Ghany. 2014. "Harvey Alter and Michael Houghton: The Discovery of Hepatitis C and the Introduction of Screening to Prevent its Transmission in Transfused Blood." In *Pioneers Of Medicine Without A Nobel Prize*, edited by Gilbert R. Thompson, 197 - 214. London: Imperial College Press.

Selzner, Nazia, and Gary A. Levy. 2020. "Hepatitis." https://doi.org/10.1036/1097-8542.314700. https://www.accessscience.com/content/hepatitis/314700.

Semeniuk, Ivan. 2020. "University of Alberta virologist Michael Houghton jointly wins Nobel Prize for medicine." The Globe and Mail. Accessed November 13, 2020. https://www.theglobeandmail.com/canada/article-canadian-virologist-michael-houghton-jointly-wins-nobel-prize-for/.

Sheikh Hamdan Bin Rashid Al Maktoum Award for Medical Sciences. "Prof. Harvey J Alter." Awards Centre. Accessed November 12, 2020. http://www.hmaward.org.ae/profile.php?id=1849.

Starr, Douglas P. 1998. *Blood : an epic history of medicine and commerce*. 1st ed. ed. New York: Alfred A. Knopf.

The American Red Cross. "Blood Needs & Blood Suply." Accessed October 23, 2020. https://www.redcrossblood.org/donate-blood/how-to-donate/how-blood-donations-help/blood-needs-blood-supply.html.

---. "History of Blood Transfusion." Accessed November 6, 2020. https://www.redcrossblood.org/donate-blood/blood-donation-process/what-happens-to-donated-blood/blood-transfusions/history-blood-transfusion.html.

Tomso, Daniel J. 2003. "DNA Libraries." *Genetics,* edited by Richard Robinson, 222-225. New York, NY: Macmillan Reference USA. https://link.gale.com/apps/doc/CX3406500076/GVRL?u=bingul&sid=GVRL&xid=d14ae13a.

Trepo, Christian. 2014. "A brief history of hepatitis milestones." *Liver International* 34 (s1): 29-37. https://doi.org/10.1111/liv.12409. https://onlinelibrary.wiley.com/doi/abs/10.1111/liv.12409.

U.S. Food and Drug Administration. 2020. "Complete List of Donor Screening Assays for Infectious Agents and HIV Diagnostic Assays." Accessed October 23, 2020. https://www.fda.gov/vaccines-blood-biologics/complete-list-donor-screening-assays-infectious-agents-and-hiv-diagnostic-assays#TOC.

University of Alberta. 2020. University of Alberta virologist awarded Nobel Prize in Physiology or Medicine. *folio*.

Virology Education. "Awards." Accessed November 16, 2020. https://www.virology-education.com/event/previous/hep-dart-2019/awards/.

World Health Organization. 2017. *Global hepatitis report 2017*: World Health Organization. (accessed October 23, 2020).
---. 2020a. "Hepatitis." WHO Newsroom Fact Sheets. Accessed October 23, 2020. https://www.who.int/health-topics/hepatitis.
---. 2020b. "Hepatitis C." WHO Newsroom Fact Sheets. Accessed October 23, 2020. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c.