ANTI-COVID-19'S VACCINES. CURRENT SITUATION (DECEMBER, 27, 2020)

In 1966, a pathogenic coronavirus was observed for the first time; twenty years before, right after World War II, a 16-years-old Scottish teenager, started a job as a laboratory assistant in the histology department of a Glasgow hospital. There, June Dalziel Hart (maiden name), learned to observe under the microscope and detect potential pathogens in the samples examined.

June Dalziel Hart was transferred to St. Thomas Hospital in London. There, she worked with a virus listed (but never observed) as B814 responsible for common colds. In addition to the microscopic study, tests were carried out on volunteers who were deliberately infected. Initially, this procedure did not seem dangerous since it was a common cold. However, the volunteers described episodes of discomfort which did not correspond to those of other colds.

On the other hand, the virus was neutralized *in vitro* by fatty solvents. This meant that unlike other viruses causing cold, B814 had a layer of lipids.

However, a crucial element was missing: there was not an image of the virus.

June Dalziel Hart began examining cells under the electronic microscope, comparing them to other cells infected with the flu and herpes viruses, as controls.

Although at the time (the 1960s) the technological sophistication of the electronic microscopy was significantly more precarious than the one these devices have today, June Dalziel Hart became renowned in their use. This is described in the book co-signed by David Tyrrell and Michael Fieder in 2002, entitled *Cold Wars: The Fight against the Common Cold* [(1)].

After World War II, the United Kingdom created in 1946, in Salisbury, the *Common Cold Research Unit*, being active until 1990. David Tyrrell directed the research effort against colds from the creation of the Unit until 1957.

The researchers at the "Common Cold Research Unit" carried out crucial basic research, succeeding in cultivating cells infected by viruses, defining many of the structural characteristics of these microorganisms, and establishing the precise mode of spread of the infection (droplets exhaled during respiration and fomites).

Some research groups of the *Common Cold Research Unit* carried out experiments beyond Salisbury: on an isolated island in the South Atlantic (Tristan da Cuhna, now part of the United Kingdom despite its Portuguese name), at the British base in Antarctica. and at Seal Island (close to the Scottish coast yet very isolated). Any contact with people outside these isolated places triggered colds among the inhabitants. The relationship, now undoubted, between stress and the incidence of colds (possibly also of many other infections) was also demonstrated.

J. Dalziel Hart (June Almeida after her marriage to Brazilian Enriques Rosalio Almeida) was able to visualize the B814 virus. A problem arose: how to name the new virus. June described it as surrounded by a halo, like a solar corona. Thus, it was named coronavirus.

This new virus (the first coronavirus discovered) was immediately linked to severe bronchitis in chickens.

Almost at the same time as of June Dalziel Hart's explorations, another researcher, Kenneth McIntosh, a paediatrics professor at Harvard University, discovered another coronavirus that also causes a mild upper respiratory infection in humans.

Until 2002, coronaviruses (their number had increased significantly since then) were not considered a threat to human health.

June Almeida died on December 1, 2007, in Bexhill, England at the age of 77. She had time to experience the first epidemic SARS-Covid-1(years 2002-2003), but not the current pandemic, caused by a type of virus not too different from the one she had first observed under an electronic microscope in 1966.

Coronaviruses acquired pathological notoriety in the 21st century: in 2002, a series of atypical cases of pneumonia that arose in the Chinese province of Guangdong spread to twenty countries. For this clinical picture, the acronym SARS (Severe Acute Respiratory Syndrome) was coined. It was a type of coronavirus that was designated as β-CoV. Mortality was around 10%, not spreading in a pandemic way since only those with active symptoms were contagious.

In 2012 another β-coronavirus emerged among camel herders in the oases of the Arabian Peninsula. The infection was designated MERS (Middle East Respiratory Syndrome). Their mortality was greater than 30%. Although isolated cases persist, the virus appears controlled.

Since December 2019 (perhaps earlier) the world has been facing a fastly-spreading coronavirus (SARS-CoV-2), declared pandemic by the World Health Organization since March 11, 2020.

Just 10 days after the first cases (atypical pneumonia) were known in the city of Wuhan, Hubei province, People's Republic of China, the complete genome of this new virus was made public; and immediately several laboratories around the world began the urgent search for potential vaccines. Currently, there are around 100 active research projects with that aim. In this paper, only those that are in an advanced stage of a pre-clinical investigation, or that have begun to be administered, will be discussed.

The authors are aware of the variability of the information that follows. It must therefore be considered as a *photograph* of the situation in the last days of the year 2020. Therefore, this text will be focused on the vaccines developed by Pfizer-BioNTech, Moderna Therapeutics, Johnson & Johnson, Oxford-Astra-Zeneca, and Sinovac.

Pfizer-BioNTech’s Vaccine. -

The German biotechnology company BioNTech developed the vaccine named BNT162b2 (Tozinameran).

Similarly, to the Moderna Therapeutics' vaccine (see later in this paper), the Pfizer-BioNTech' vaccine is designed from the *genetic instructions* for the synthesis of the protein S (S, of Spike) which studs the coronavirus spheroid. The vaccine uses messenger RNA embedded in lipid nanoparticles. This sort of RNA is a labile molecule, and therefore the vaccine must be preserved and distributed under extreme refrigeration conditions (-79º Celsius or -110º Fahrenheit).

Preliminary studies showed that the vaccine offers excellent protection for at least 10 days after the first dose. A second dose administered 21 days later, will strengthen, and prolong the protection for a still undetermined period.

Each vial of the vaccine contains 5 doses of 0.3 ml. The vial should be thawed, reconstituted with 0.9% saline. The reconstituted vial must be used within no more than 6 hours.

The German biotechnology company BioNTech began working on the development of the vaccine in January (2020), two months before the World Health Organization pandemic declaration (March 11, 2020). In March, the US multinational pharmaceutical Pfizer and the German biotechnology company BioNTech signed collaboration agreements.

In May, clinical trials (phases 1/2) of two versions of the vaccine, both designed with viral messenger RNA, started. Of the two versions, the one designated as BNT162B1 was selected for its better tolerance.

On July 22, the Trump Administration signs a contract of 1,900 million dollars for the future acquisition of 100 million doses, expandable to another 500 million, if, as it has happened, the Food and Drug Administration wrote a favourable report for its approval.

On July 27, a combined clinical trial begins (phases 2/3) with the participation of 40,000 volunteers from various countries (United States of America, Argentina, Brazil, and Germany).

On September 12, Pfizer-BioNTech announced the extension of their clinical trial in the United States.

On November 9, the first (provisional) results were known, communicating the effectiveness of approximately 90%, without significant adverse effects. Days later, after the publication of the first results of the Moderna Therapeutics vaccine, Pfizer announced the protection of around 95%.

Pfizer-BioNTech requests (November 20) approval to the FDA, under *emergency status*.

On December 2, the UK grants emergency clearance to the Pfizer-BioNTech vaccine. It thus becomes the first Western country to authorize the administration of an anti-COVID-19 vaccine. The approval is restricted to health workers and people aged over 80.

A few days later, on December 9, Canada grants the authorization; and the next day (10) Saudi Arabia does.

A day later (December 11), following a favourable report from the FDA, the United States authorizes the vaccine, followed by Mexico.

On December 14, vaccination begins in the United States; and on December 27, in the European Union.

Pfizer expects to manufacture 1.3 billion doses in 2021. Remember that the degree of protection reported is only achieved after the injection of two doses.

Moderna Therapeutics' Vaccine. -

Moderna Therapeutics' vaccine has been developed in close collaboration with the National Institutes of Health in the United States. It is designated as mRNA-1273. As inferred from its designation, it also uses messenger RNA which encodes the synthesis of the S protein of the SARS-CoV-2. Its conceptual design is similar to the one developed by Pfizer-BioNTech: it is administered as lipid nanoparticles that encompass the messenger RNA for the protein S synthesis. The first results reported the protection of approximately 94%. It has an important advantage compared to its Pfizer-BioNTech counterpart: it requires less demanding refrigeration conditions, needing to be conserved between -4 and -20º Celsius (equivalent to the range + 24º to -4º Fahrenheit). If kept under such conditions, its effectiveness can be preserved for no less than 1 semester, according to its manufacturer.

Protection (94% according to Moderna Therapeutics) is achieved after the administration of two doses separated by 28 days. The first dose achieves a weak immunity which is strengthened with the administration of the second one. Studies confirm that protection of this vaccine extends for at least one trimester.

Each vial contains 10 doses of 0.5ml. The vials should be warmed to room temperature (approximately 25 ° Celsius) before administration.

Moderna Therapeutics began developing its vaccine in January (2020), two months before the World Health Organization pandemic declaration, and its test in humans started just five days after it (March 16, 2020). That same day, the federal government of the United States (through Operation Warp Speed [2]) granted 483 million dollars to Moderna Therapeutics conditioned on the supervision of the research process, including clinical trials, by the National Institutes of Health.

On July 27, following limited clinical studies (phases 1/2) Moderna Therapeutics and the National Institutes of Health began a phase 3 clinical trial involving 30,000 American volunteers, of whom a quarter were 65 years or older.

On July 28, the laboratory communicates that its vaccine protects apes against the SARS-CoV-2 coronavirus.

On August 11, the US government makes a conditional acquisition of 100 million doses for 1,500 million dollars, subject to the FDA approval which, at the time of the writing of this article, has been already made effective.

On November 12, the laboratory published the preliminary results of phase 3 (3) clinical trial that stated protection of 94.1%, substantially higher than initially expected by the researchers.

On November 30 Moderna Therapeutics requests *emergency approval* from the FDA.

On December 2, the laboratory registers a clinical trial to test the vaccine in children between 12 and 18 years of age (4).

Finally, on December 18, the laboratory receives authorization for the vaccine, which begins to be injected on the 21st.

Moderna Therapeutics expects to manufacture one billion doses throughout 2021. Protection with this vaccine requires two administrations spaced one month apart.

Oxford-Astra-Zeneca's Vaccine. -

In January 2020, the Jenner Institute of the University of Oxford began researching a vaccine against what seemed then an emerging type of pneumonia with the risk of acquiring (as it has been) a pandemic dimension. The British institution partnered with Astra-Zeneca to develop a vaccine designated ChADOx1 [(6)] (also named AZD1222). The first results stated protection of 90%, but recent events have clouded its prospects.

The Oxford-Astra-Zeneca vaccine uses double-stranded DNA (instead of messenger RNA). The DNA (which contains the information for the synthesis of protein S) is inserted into an adenovirus which acts as a vector to carry out the DNA into the cell nucleus. Adenoviruses are usually responsible for common colds, so a genetically modified chimpanzee adenovirus (ChAdOx1) has been used in this vaccine, in such a way that it cannot replicate inside the cell.

This AZD1222 vaccine is the result of years of research for other potential applications.

Adenovirus-based vaccines have enabled Johnson & Johnson to develop a vaccine against the Ebola virus [(5)]; and others against HIV (pre-clinical phase) and Zika virus (very preliminary investigations), are under development.

DNA (unlike messenger RNA) is a molecule more resistant to hydrolysis. Hence, the DNA-using vaccines are less demanding in their conservation: between 2 and 8º Celsius (35º to 46º Fahrenheit).

On March 27 the first tests on human volunteers began. A combined trial (phases 1/2) began in the UK on April 23, and seven days later (April 30) the University of Oxford established a collaboration agreement with Astra-Zeneca for the next stages of vaccine development.

On May 21, the United States government granted 1.2 billion dollars to Astra-Zeneca, under the umbrella of Operation Warp Speed.

On May 28, the clinical trial combining phases 2 and 3 began in the United Kingdom. During the study, some participants received the first dose with half the amount planned.

On June 23, the phase 3 clinical trial begins in Brazil; and on the 28th of the same month, a combined clinical trial (phases 1/2) begins in South Africa.

On June 30, an article was published in Nature [7] which certifies the safety of the vaccine in experimental animals, and its efficacy in the prevention of severe pneumonia.

On August 18, a phase 3 clinical study begins in the United States in which 40,000 volunteers participate.

On September 6, clinical trials are suspended worldwide (except in Brazil) after the notification of a serious adverse reaction (two, according to some information) of transverse myelitis.

After the pertinent analysis, the clinical trial was resumed in the UK on 12 September; and on the 23rd in the United States, after a favourable report from the FDA.

On November 23, Astra-Zeneca announced that an analysis of the subgroup of volunteers who were administered, by mistake, with a first dose which had half of the programmed units, attained greater protection than those who received the planned dose (90% *vs* 62%).

On December 7, the Serum Institute of India announces the request for approval of the Astra-Zeneca vaccine, registered in the Indian Federation as Covshield®.

On December 8, Oxford-Astra-Zeneca published [8] in The Lancet the first results of phase 3 clinical trial.

On December 11, Astra-Zeneca announced a collaboration agreement with the Russian company that manufactures the vaccine Sputnik-V, which has also been developed using adenovirus technology.

Recently, another collaboration agreement has also been known with the main Chinese vaccine manufacturer (Shenzhen Kangtai Biological Products) to produce its vaccine in the People's Republic of China, both for its internal use and its export to other countries. Besides, the mentioned Chinese company will also manufacture an anti-COVID-19 vaccine of its design.

In 2021, Astra-Zeneca (together with subsidiaries) expects to manufacture up to two billion doses, each person needing to receive two of them over a month.

Johnson and Johnson’s Vaccine. -

Janssen Pharmaceutica, a Belgian division of Johnson & Johnson, in collaboration with Beth Israel Deaconess Medical Centre, is investigating a vaccine designated as

78436735 (or: Ad26.COV2.S). Results of the ongoing clinical trial are expected in January 2021.

Similar to one developed by Oxford-Astra-Zeneca, it is a DNA vaccine that is administered encapsulated into an adenovirus.

Being a DNA-based vaccine, freezing is not required for its preservation, only needing to be kept between 2º and 8º Celsius (35º to 46º Fahrenheit).

Johnson & Johnson began researching the vaccine in January, receiving in March 456 million dollars in funding from the US government through Operation Warp Speed.

In July, the combined clinical trial [(9)] (phases 1/2) began, using a single dose per person.

In August, the United States' government signed an agreement committing to a purchase of 100 million doses for one billion dollars, conditioned on the authorization of the vaccine.

In September, Johnson & Johnson begins the phase 3 clinical trial.

The European Union signs on October 8 the purchase of 200 million doses.

On October 12, the clinical trial was stopped due to an adverse reaction ("inexplicable illness"), resuming on the 23rd.

A second clinical trial [(10)] of roughly 45,000 participants began on November 16 to assess the possible advantage of administering two doses of the vaccine, instead of one.

According to its schedule, Johnson & Johnson plans to request approval in January 2021.

Sinovac's Vaccine. -

The Chinese company Sinovac developed an anti-COVID-19 vaccine called CoronaVac®. Preliminary results of its efficacy are expected for January 2021, although, during its phase 3 clinical trial in Brazil, an efficacy of more than 50% was anticipated, which is the threshold whose meeting the FDA announced as sufficient for the approval of any vaccine.

For the design of CoronaVac®, the researchers used tissue samples from patients infected with the virus from various countries: The People's Republic of China, the United Kingdom, Italy, Spain, and Switzerland. A sample from patients from China was finally used to manufacture the vaccine.

Monkey kidney cells were infected with coronavirus, treating the samples with *propriolactone*. This substance inactivated the viruses in the tissue samples. The genes of the coronavirus were rendered useless, but not the proteins (including the S protein) which served as antigen to an antibody response.

The inactivated viruses were mixed with small amounts of aluminum which acts as an adjuvant to enhance the immune response.

Sinovac began developing its vaccine in January (2020). In June, the combined clinical trial (phases 1/2) began with 743 volunteers, with no adverse effects reported. A phase 3 clinical trial began in Brazil in July, followed by others in Indonesia and Turkey. The vaccine has been approved for its use in China and other countries, although with restrictions.

The first results of the combined clinical trial (phases 1/2) showed that the vaccine achieved a modest production of antibodies. Results of the phase 3 clinical trial are expected around January 2021.

Until now it was believed that coronavirus mutations were rare, with minimal effects in their pathogenicity. To learn more about this topic, it is highly recommended to read a lecture, freely accessible online, delivered in 1988 by Joshua Lederberg (1958 Nobel Prize in Physiology or Medicine *ex aequo* George Wells Beadle and Edward Lawrie Tatum), entitled *Pandemic as a Natural Evolutionary Phenomenon*. The text theorizes about the Darwinian model in the evolution of the microbial underworld.

While this text is being written, there has been news of two mutations, arising in the south of England and South Africa, that seem to increase the contagiousness of the coronavirus but not its pathogenicity, but, according to the current knowledge, do not affect the efficacy of vaccines. However, widespread vaccination puts *selection pressure* in favour of the emergence of resistant strains [(11)].

It is practically impossible to know the evolution of this pandemic virus [(12)]. The effect of the different vaccines on its epidemiology, the prospects of the acquisition of herd immunity, as well as the scope of the long-lasting socio-economic changes it will produce, remain unknown.

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