Memoirs and Communications
Of members and correspondents of the Academy

PHYSIOLOGIC CHEMISTRY: Of infectious diseases, especially the disease of chicken cholera, by **Mr. Pasteur**.

Infectious diseases consist most of the major disasters, such as small pox, scarlet fever, rubella, syphilis, glanders, anthrax, yellow fever, typhus, and bovine plague. This list is already quite charged and yet incomplete. All of pathology is represented here.

As long as the ideas of Liebig on the nature of ferments ruled, the microbes were considered as substances ascribed to an intestinal movement capable of infecting tissues of the host organism and transforming them to a microbe of the same nature. Liebig recognized the similarities between the ferments' appearance, multiplication and ability to decompose and the phenomena of life. He claimed through his introduction to his " Treaty of Organic Chemistry" that this is a false illusion.

All my experiences that I have presented to this Academy over the past 23 years have demonstrated, either directly or indirectly, that the opinions of Liebig are false. A unique method has guided me to study microscopic organisms. It essentially consists of culturing these organisms purely, without the coexistence of heterogeneous dead or live substances. By using this method, several questions have been answered simply and decisively. One of my first applications of this method was in 1857-1858. Liebig said that the all ferments are nitrogenous products of the organism, such as fibrin, albumin, casein, etc. that have been altered upon contact with air. All recounted fermentation processes described these materials as present or active. The origin and progress of fermentations and diseases were believed to be spontaneous. In order to prove that the hypothesis of this German scientist was false, I mixed artificial media containing exclusively pure water and mineral substances necessary for the life of fermented substances with the germs of the ferments of these diverse substances. Under these conditions, fermentation was accomplished regularly and purely, which could not be the case in spontaneous fermentations of nature. After the elimination of all albuminoid substances, the ferment appeared as a live being that used, in all of its successive generations, carbon from the fermented substance, and nitrogen, phosphorus, potassium and magnesium from the mineral medium. The assimilation of these elements is a necessary condition for the formation of any small or large being.

Consequently, the theory of Liebig does not have any foundation and the phenomena of fermentation are presented as simple phenomena of nutrition accomplished in exceptional conditions, most importantly, in the absence of air contact.

Both human medicine and veterinarian medicine used the light of these results. We then started researching if the microbes and infectious elements are animated beings. In 1863, Dr. Davaine studied the functions of the anthrax bacteria that he discovered back in 1850. In 1868, Dr. Chauveau tried to establish that virulence is due to solid particles found in all microbes. In 1872, Dr. Klebs attributed traumatic microbes to microscopic organisms. In 1876, through the method of cultures, Dr. Koch found the spores of the septic bacteria to be very similar to the ones I demonstrated in 1865-1870. Therefore the etiology of several diseases was attributed to the existence of microscopic ferments. Today then, the most resistant minds to the doctrine of germs are destroyed, but obscurity still covers several points of the truth.

For the majority of virulent diseases, the microbe has not yet been isolated through cultures or demonstrated to be alive, making these unknowns of pathology the causes of mysterious morbidity. In addition, the natural history of diseases caused by these microbes presents extraordinary circumstances, such as the absence of recurrence. One is barely able to present a hypothetical explanation of non-recurrence based on any experiments. Isn't it surprising to observe that vaccinia, a virulent but benign disease, protects against itself and small pox, a more serious illness? The practices of vaccination and variolisation have been known in India for the longest time. Even before Jenner demonstrated the efficacy of vaccinia, people of the countryside where he practiced already knew that cowpox protected against small pox. The facts about vaccinia are unique, but the facts about non-recurrence of virulent diseases are more general. The organism never expresses twice the effects of chicken pox, scarlet fever, typhus, plague, small pox, syphilis, and others, as the immunity persists for a long time at least.

Although one is obligated to have the greatest humility facing these mysteries, I dare to think that the Academy will see in the facts that I will present some unexpected revelations about the study of virulent diseases.

Chicken cholera is a disastrous disease seen in the farmyards. The affected animal is weak, waddling with fallen wings. The feathers are erect giving the animal the form of a ball. An invincible somnolence overcomes it. If the animal is forced to open its eyes, it seems to awaken from a deep sleep but afterwards later the eyelids close again. Often the animal dies after a silent agony, before it had a chance to move. Sometimes, the animal shakes its wings for a few seconds before dying. The animal's internal disorders are considerable. According to Zundel's dictionary, the disease is caused by a microscopic organism that was described by Mr. Moritz, a veterinarian in high Alsace, and then better illustrated by Mr. Peroncito in 1878, a veterinarian in Turin. Finally, this small organism was cultures and isolated in neutralized urine in 1879 by Mr. Tossaint, a professor at the veterinary school of Toulouse, and proven to be the cause of virulence in the blood.

The first and most useful condition to be fulfilled in the study of microscopic parasitic diseases is to obtain a liquid medium where the infectious organism can be easily cultured and isolated from other organisms of different species. The neutralized urine used to repeatedly culture the bacteria of Davaine and demonstrate that it was the microbe of anthrax (1877, Pasteur and Joubert) does not fulfill in this instance the double goal needed. A good culture medium for the survival of the chicken cholera microbe is a chicken muscle broth, neutralized in potassium and sterilized by heating it to more than 100 degrees (110-115). The microscopic organism multiplies very easily in this culture medium. In few hours the clear broth becomes turbid and full of small thin dark particles, with a central strangulation, that resemble isolated points. As these particles do not move, they must necessarily belong to a different group from that of microbes. These particles remain for now of unknown nature but should be placed close to microbes once we succeed in culturing them.

The culture of microbes presents interesting peculiarities.

One of the culture media I successfully used in my previous studies is the water of **levure,** which consists of beer **levure** and water filtrated to a perfect clear state then sterilized by heating it up to 100 degrees. Several microscopic organisms adapt themselves to the nutrition provided by this liquid, especially if it is neutralized. Although this medium allows the anthrax bacteria to grow within hours, it is not suitable for the growth of the chicken cholera microbe that promptly dies in it in less than 48 hours. This is analogous to the fact that a microscopic organism can be harmless to a certain animal species if it is unable to grow in the host organism or to reach the essential organs for its development.

The sterility of the **levure** water medium when inoculated with this microbe is a good control to test for the purity of this organism's culture in chicken broth. The **levure** water remains clear and sterile of the culture is pure and becomes turbid if impurities grow.

Another peculiarity about the chicken cholera microbe is that it does not kill turkeys when inoculated with it, contrary to what is observed with chicken. Turkeys of a certain age show a local lesion at the site of inoculation, which ends up as a more or less large abscess. This abscess opens up spontaneously then closes again and heals without the animal showing any sign of illness. These abscesses persist for several weeks surrounded by a pyogenic membrane and are filled with creamy pus, full of multiple microbes and white blood cells. The microbe multiplies in the abscess resembling a closed vase, remains viable in a pure state and can be recovered, without sacrificing the host animal. To prove the above, inoculating the chicken with some of this pus kills them rapidly, while inoculating the turkeys that provided the microbe does not affect them. We are hereby witnessing the local evolution of a microscopic organism that leads to an abscess formation, without affecting or killing one animal species, while still capable of killing

animals from another species. The microbe is also capable of killing the animal in which it exists in an abscess form if it contaminates the host's blood or internal organs. Chicken and rabbits living with infected turkeys might suddenly become sick and perish, without the health of turkeys being affected. The above phenomenon is observed if the turkeys abscesses open up and some of their pus spills over the chicken and rabbits food. Observing the death of rabbits and chicken without an apparent cause would lead to believe in the spontaneity of the illness, especially that one would not suspect the source of the illness to be from the apparently healthy looking turkeys. Similarly, many mysteries in the history of infectious diseases will, one day, be resolved as the one just described. We may refute theories that are contradicted by facts, but not ones that we do not understand, as the combinations of nature are simpler and more varied than our imagination.

To support the above facts presented, I should mention that a few drops of the microbe's culture added to the bread or meat fed to chicken allow the microbe to multiply abundantly in the animals intestinal canal and infect their feces. These infected feces are able to kill individuals when inoculated with them. These facts show the ease with which this grave disease propagates in the farmyard. Evidently, the excretions of sick animals play a major role in spreading the disease. Therefore, the easiest way to stop the disease is to isolate the animals for a few days, flush the farmyard with water, especially acidified water with sulfuric acid that easily destroys the microbe, eliminate the waste then reassemble the animals. Consequently, all causes of infectivity would have been abolished, as, during isolation, affected animals would have already succumbed to this devastating disease. Subjecting the microbe to serial cultures in chicken broth, by successively transferring infinitely small inoculants from one culture to the other, does not diminish the virulence of this microscopic organism. This parallels the ease of multiplication of the microbe inside the body of birds (Gallinaceans*). The microbe is extremely virulent as a minimal fraction of a drop of a culture causes death in 20 over 20 cases in 2 to 3 days, and more often in less than 24 hours.

With this, I now present the most important facts of this communication.

We can diminish the microbe's virulence by changing the mode of culturing. This is the crucial point of my subject. I ask the Academy not to criticize, for the time being, the confidence of my proceedings that permit me to determine the microbe's attenuation, in order to save the independence of my studies and to better assure their progress.

The decrease in virulence is manifested by a delay in the development of the microbe in cultures. Basically, the microbe has two varieties: in its first state, it is very infectious, and, when inoculated, it can kill in 20 over 20 of cases, and in its second state, it causes the illness in 20 over 20 of the cases but does not lead to death of the host organism. These facts are important as they permit us to understand the disease's recurrence or non-recurrence. If we start with 40 chicken and inoculate 20 of them with a virulent microbe,

they will all die, if we inoculate the remaining 20 with the attenuated microbe, all will become ill but none will die. If we reinoculate these 20 remaining chicken, after recovery, with the virulent microbe, they will not die. Evidently, the disease is self preserved and has the characteristics of virulent diseases that do not recur.

Let us not be astonished by the peculiarity of these results as this is not the first time such phenomena are described. These facts do however have a novelty that we shall extrapolate later. Before and during the days of Jenner, the practice of inoculating with small pox to prevent the disease was widely spread. In several countries nowadays sheep are inoculated with sheep pox to protect them from it, and similarly, the bovine species is inoculated with peripneumonia to protect it from this serious disease. The chicken cholera offers a similar example of immunity, which is an interesting fact but not a novel principle. The real novelty of the above observations is in revealing the cause of the illness as a microscopic parasite, a living being, able to be cultured. The nature of small pox, vaccinia, glanders, syphilis and plague microbes remains unknown. The new microbe is an animated being and the disease it causes is non-recurrent, a characteristic common to all the virulent diseases due to microscopic parasites. The microbe's existence bridges the gap between the virulent illnesses caused by a live microbe and those caused by a non identified one.

It is not in my intention to make you think that these facts present the mathematical clarity and regularity that I emplied, as it would undermine the variability of animals constitutions and the general manifestations of life. The chicken cholera microbe does not always kill in 20/20 of the cases, but from what I have seen, it kills at least in 18/20 of the cases. Similarly, the attenuated form does not always conserve life in 20/20 of cases but it conserved it at least in 16 to 18 over 20 cases. The recurrence of the disease cannot be absolutely prevented after one inoculation of the attenuated microbe but surely is after two inoculations.

In analogy to the results obtained with vaccinia in relation to small pox, the attenuated microbe acts similarly as a vaccine against the killing microbe by producing a benign illness and therefore protecting from the fatal form of the disease. In order to make this attenuated microbe a true vaccine, comparable to that of cowpox, it should be fixed in its proper form and be easily recovered from the original preparation when needed. This reflects the fear that preoccupied Jenner for a while when he demonstrated that the inoculation of cowpox protected against small pox, as he believed that one should always refer back to the cowpox microbe to prevent the disease. The major difference in the case of chicken cholera is the fact that we know our vaccine is a live being. Jenner soon recognized that he could dispense of the cowpox of the cow and transmit the vaccine from arm to arm. We can make a tentative analogy by transmitting our live microbe from culture to culture. One is surprised to find that the microbe does not regain its active virulence and keeps its attenuated form. After a small number of serial cultures, the virulence was not increased, and we can consequently believe that we are dealing with a

true vaccine. Moreover, one or 2 experiments support the idea that the attenuated microbe is conserved as if present in the turkey's body. Several further experiments are still needed to demonstrate that the attenuated form is conserved after several cultures and several inoculations.

In summary, we are in the possession of an illness, produced by a microscopic parasite, that can become non-recurrent despite its parasitic characteristic. We also know that a variety of the microbe protects against the illness, as does the vaccine against small pox.

I now digress to an interesting point. We conclude from the above facts that one can easily obtain chicken affected by chicken cholera without having the disease kill them, and one can assist in their recovery. As far as I know, clinical surgery has never witnessed phenomena more strange than the manifestation of complete recovery following pectoral muscle inoculation. The microbe multiplies in the thickness of the muscle as it would in a vase. The muscle hardens, thickens and develops a white surface as it becomes studded with pus globules, without suppurating. The muscle's histology becomes distorted as islets of the microbe seed it, alter it and feed on its substance. I will later present colored figures showing the curious disorders caused by the microbe in the case of recovery. The microbe's development is slowly arrested and it disappears, while the necrosed section of the muscle reassembles, hardens and collects in a cavity. The necrosed part eventually forms a sequestrum, isolated in the cavity, palpable through the skin inside or on the surface of the muscle, that can be incised and extracted. Following extraction, the small incision made in the skin heals immediately and the now empty cavity fills up with repaired muscle elements. I will show the Academy some of these demonstrations……

I am eager to finish with a legitimate explanation of the non-recurrence of this virulent illness. When we reinoculate a repeatedly vaccinated chicken with the weakened microbe, a local lesion appears that seems insignificant when compared to the ones obtained with the initial inoculations, and especially the first one. The reason behind the difference in these lesions resides entirely in the relative ease of development of the microbe after the first inoculations, and in a null, weak or promptly arrested development after the last one. The conclusion of these facts is striking: the inoculated muscle, that was initially very sick, has become, after healing and repair, incapable of cultivating the microbe. It seems as if the initial microbe inoculations have depleted in the muscle a certain element that healing does not reconstitute and that the absence of which hinders the development of this small organism. This explanation will, without doubt, become general and applied to all infectious diseases.

I would like to point to the Academy two main consequences to the facts presented: the hope to culture all microbes and to find a vaccine for all infectious diseases that have repeatedly afflicted humanity, and are a major burden on agriculture and breeding of domestic animals.

In conclusion, it is my duty and pleasure to add that I witnessed, in these delicate and long studies, the dedication and intelligence of Mr. Chamberland and Mr. Roux.

Notes:
*glanders: fatal disease of horses transmitted to humans, producing ulceration of the nostrils.

*Gallinaceans: omnivorous birds like turkeys, chicken.

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