

In the course of an examination of American refined lead, containing comparatively large quantities of copper and nickel, the bismuth had been precipitated in the ordinary manner by an excess of sodium carbonate, from the potassium cyanide solution of the 5th group of metals (Fresenius). In the alkaline filtrate, H_2S produced a reddish-brown precipitate, which could not be identified as the sulphide of any of the well known heavy metals. It was readily soluble in dilute nitric acid. From this solution a basic salt was precipitated by water. Potassium carbonate produces a precipitate which is soluble in an excess of the boiling precipitant. Hydrogen sulphide precipitates the original sulphide from the alkaline solution.

The quantity of sulphide was very small, amounting to about 1 mgrm from 200 grms of lead.

The reactions, as far as they could be verified, considering the smallness of the quantity of material, correspond to the description of norwegium as given by Dahill.

The refined lead was a mixture of leads from various sources, but it is not improbable that there may be a connection between the presence of copper, nickel and norwegium. Proper attention given to the various lead ores, especially those which yield lead containing larger quantities of copper and nickel, will undoubtedly throw further light upon this subject.

The results obtained seemed of sufficient interest for publication for the benefit of those who may be more favorably situated, both with regard to time and material, than I am at present.

XXVI.—ON CHICKEN CHOLERA: STUDY OF THE CONDITIONS OF NON-RECIDIVATION AND OF SOME OTHER CHARACTERISTICS OF THIS DISEASE.

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In the communication which I had the honor of presenting to the Academy in the month of February last, I announced, among other results, that chicken cholera originates in a microscopical parasite; that there is an attenuated virus of this disease, and that one or more inoculations of this attenuated virus may preserve chickens from death when inoculated with the virus of maximum virulence. On account of the striking similarity that these two forms of virus present with the effects of variola and vaccine in man, it becomes in-

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teresting to ascertain not only if the immunity from the more aggravated form of virus is absolute, for the regions of the body which have undergone the preventive inoculation, but also if this immunity exists in the system, no matter what portion of the animal may have been inoculated, and what may have been the manner of introducing the virus.*

To explain with brevity the results which I have to communicate, I may be allowed to use the word *vaccinate*, to express the act of inoculating a chicken with the attenuated virus. This being admitted, I may state, as the result of many experiments, that the effects of vaccination are very variable. Some chickens are little affected by the most virulent virus after one inoculation of the attenuated virus; others require two such inoculations, and even three. In every case, the preventive inoculation does some good, because it acts in a certain measure. Vaccination, then, may be of several degrees; but we may always succeed in completely vaccinating a chicken, which means that we can bring it to such a condition that it becomes incapable of being affected by the most virulent virus.

To make this matter clear, I will now give the results of experiments: I take eighty new chickens (I call *new* those which never suffered before with chicken cholera). On twenty of these, I inoculate the most virulent virus—and they all die. Of the sixty that remain, I take another lot of twenty, and I inoculate them with that quantity of the most attenuated virus which the point of the needle will take up †—and not one dies. Are they then vaccinated for the aggravated form of virus? Some are and some are not, for if I afterwards inoculate these twenty chickens with the most virulent virus, six or eight of them will not die, although they may be ill, while in the first case every inoculated chicken died. I take again from the remaining chickens another lot of twenty, and these are vaccinated with the attenuated virus exactly as the preceding lot, and, a week afterwards, they are again vaccinated in the same manner. Are they now safe from the virulent virus? We now inoculate these twenty chickens with this virulent virus, and, instead of there being six or eight which do not die, there are twelve or fifteen. Finally, I take the twenty remaining chickens, and vaccinate them suc-

* From all I have seen and read of vaccine in man, and from my experiments on chicken cholera, I infer that *vaccine* rarely acts as a complete preventive. There are cases cited, of vaccinated persons who have had the variola, and there are even cases of persons who have had it, afterwards, as much as three times.

† There are degrees of attenuation as well as of virulence. I will give explanations in a future communication.

cessively three or four times. If now I come to inoculate them with the most virulent virus, not one will die. In this case, chickens are brought to the condition of animals who are incapable of suffering from chicken cholera.

As to the cause of non-recidivation, I find it impossible to resist the idea that the microscopic germ, which causes the disease, finds in the body of the animal conditions suitable to its development, and that to satisfy the necessities of its life, the germ alters certain substances, or destroys them, which comes to the same thing, whether it assimilates them, or whether it consumes them with oxygen borrowed from the blood.

When complete immunity has been reached, the most virulent germ may be inoculated into any of the muscles without producing any effect. This means that the cultivation of the germ has become impossible in these muscles. They no longer contain food for the germ.

It is impossible to convey the impression that one receives from observing such phenomena. Here are twenty chickens which never had this disease. I inoculate them in their pectoral muscles or, still better, in the muscle of the thigh, so as to observe with greater ease the effect of the inoculation. The next day all the chickens are lying down; they are very lame and seem overcome by sleep. The inoculated muscle becomes of enormous size and is profusely filled with the parasites. From time to time, a chicken dies, and, at the end of forty-eight hours, they are all dead. We may take also twenty chickens, previously vaccinated several times, and inoculate them at the same time as the others, with the same virus, in equal quantities. The next day and the next, they are all alive and in good health; they eat and cackle as usual; the cocks crow; the inoculated muscles present nothing abnormal. There is not even a sign to show where the skin was punctured. This healthy condition remains permanent.

We may now inquire whether the impossibility of cultivating the parasite is not limited to the muscles which have been inoculated. This may be answered by introducing the deadly virus in the blood vessels and in the digestive organs. I have taken ten chickens, never before inoculated, and ten others inoculated several times with the mild virus. I have then injected the worst form of virus in the jugular vein of all these chickens. The first ten have died rapidly; many of them within twenty-four hours. The ten vaccinated chickens, on the contrary, have only been slightly ill from the

incision of the skin and of the jugular vein, and were soon in good health. This shows that the blood of these ten chickens was itself *vaccinated*, which means that previous cultivation had deprived it of the materials fit for further developments of the germ.

As to the introduction of the parasite in the digestive organs, I have imitated the epidemics which depopulate poultry yards, by introducing the parasite in the food of the chickens. On the 11th of March, I brought together twelve chickens, bought at the market that very morning, and twelve others, previously vaccinated several times. Every day I gave to these twenty-four chickens a meal of the diseased muscles of chickens, who had died from chicken cholera. Through the combs of the twelve chickens which had not been vaccinated, I passed a platinum wire, so as to distinguish them from the other twelve. On the next day, the unvaccinated chickens began to sicken and die. On the 26th of March, the experiment terminated. Seven of the chickens that had not been vaccinated have died, and a *post mortem* examination reveals the fact, that the disease was introduced in the system, either through the first portion of the alimentary canal, or more frequently, through the bowels, which were highly inflamed, and sometimes ulcerated, in a manner which recalls the lesions of typhoid fever.* The five other unvaccinated chickens are more or less ill, one seriously so. As to the twelve vaccinated chickens, not one has died, and to-day † they are all alive, and in good health. We may now sum up the results as follows :

It is the life of a parasite, in the interior of the body, which causes the disease known as chicken cholera and which causes death by this disease. When the cultivation of this parasite cannot take place in the body of a chicken, the disease does not show itself. The chicken is then in the constitutional condition of animals which chicken cholera cannot attack. Animals, in this condition, may be said to be born vaccinated for this disease, because the fœtal evolution has not placed in their bodies the proper food of the parasite, or because substances, which could serve as such food, have disappeared while they were yet young. We must not wonder that there are constitutions more or less apt to receive inoculations of certain kinds of virus, for, as was announced in my first note, the broth of beer-yeast is entirely incapable of supporting the life of

* The blood is full of parasites, and the interior organs are frequently covered with pus and false membranes, particularly next to the intestinal pockets, through which the germ seems to have penetrated.

† April 26th.

the parasite of chicken cholera, while it is well adapted to the cultivation of a multitude of microscopical germs, notably of the bacteria of carbuncular disease.

The explanation to which we are led by the facts already mentioned, of the different degrees of constitutional resistance of some animals, as well as of the immunity which chickens acquire by preventive inoculations, must seem a natural one, if we take into consideration, that every cultivation modifies the medium in which it takes place. In the case of ordinary plants, the soil is modified; in the case of parasites, the animals and plants on which they live are also modified. The same thing happens with the liquids in which they live, in the case of ferments and other microscopical germs. The modifications which take place have this character in common, that new cultivations of the same species in these media soon become difficult or impossible. If chicken-broth is used for cultivating the germ of *chicken cholera*, and if, after three or four days, the liquid is filtered, to separate all the germs, and furthermore, if after this, fresh quantities of the germs are placed in the filtered liquid, it will be found incapable of producing the feeblest development. Perfectly limpid at first, the liquid remains indefinitely limpid.

We are led to believe that the cultivation of the attenuated virus in a chicken, places its body in the same state as that of a liquid which can no longer sustain the life of the germ of disease. We may extend the comparison still further, for, if we filter the broth on the second day of the cultivation, instead of on the fourth, the filtered liquid will still permit the cultivation of the germ, but less readily than at first. This may enable us to understand that the cultivation of the attenuated germ in the body of a chicken may not have removed all the food for the germ. The remainder may allow a fresh cultivation of a feebler kind. This is the same as a first *vaccination*. Subsequent inoculations will remove progressively all the materials for the cultivation of the parasite. Through the action of the circulation, a time will come when any new cultivation on the animal will remain unproductive. Then the disease cannot recidivate, and the subject becomes perfectly vaccinated.

It may seem astonishing that the first cultivation could have stopped before all the food of the germ has been destroyed; but we must not forget that the germ is aerobian,* and that, in the body of an animal, it does not find the same conditions as in an artificial

* Pasteur divides germs and other microscopic organisms into *aerobians* (requiring air to live) and *anaerobiac* (which do not require air).—*Translator*.

medium of cultivation, in which there are no obstacles to its propagation. In the body, on the contrary, it finds opposition from the cells of the organs, which are also aerobian, and are continually absorbing oxygen.

We might also account for the fact of non-recidivation by admitting that the life of the germ, instead of destroying certain substances in the body of an animal, on the contrary, adds other substances which act as an obstacle to its further development. The history of the life of these inferior beings, of all beings in fact, authorizes this supposition. The excretions due to vital functions often prevent vital functions of the same nature. In some fermentations, antiseptic products are formed while fermentation is going on, and even by the action of ferments, and these products put an end to further action, even if there are still substances left capable of undergoing fermentation. In the cultivation of our germ, there might, in the same way, be substances formed whose presence might explain non-recidivation and vaccination.

Our artificial cultivation of the parasite will enable us to examine this hypothesis. If we prepare an artificial cultivation of the germ of chicken cholera, we may evaporate the liquid in vacuo while cold, then bring it back to its original volume by the addition of chicken broth. If the extract contains a poison which destroys the germ, and if the presence of this poison is the cause of its non-development, the cultivation of the germ cannot take place in this liquid. On the contrary, the development does take place without difficulty. We cannot then believe that, during the life of the parasite, there are substances produced which prevent its further development. This is a corroboration of the opinion which we have expressed on the cause of non-recidivation in certain virulent diseases.