Serum against the Epithelioma or Diphtheria of Birds

by

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Concerned for more than ten years with the study of various diseases, caused by filterable germs, of more special interest to veterinary medicine, I became convinced that these germs form a quite homogeneous group, the important characters of which are to be found in the conditions of parasitism and may be considered in a synthetical manner in the following way:

1º. All filterable germs are obligatory parasites, that is may only manifest vital activity when inside a host, although able to remain, in a state of latent life, outside the organism. The cultures, which may sometimes be obtained in special culture media, are short-lived and therefore are not to be found amongst the collections of the most prominent institutes of research however complete they may be.

2º. They are very exacting as to the choice of their host: hog cholera is exclusively confined to porcines, foot-and-mouth disease can be transmitted easily only to artiodactyla, and contagious epithelioma even presents difficulties with regard to its transmission from one species of birds to another.

3º. In the cases were they are not observed to be so particular (rabies, cow-pox etc.) the germs are more liable to undergo mutations: *virus fixe*, vaccines, etc. The race thus obtained has vaccinating properties, and no other method for obtaining vaccines can be applied, seeing that the methods used for attenuating bacteria usually prove very unsatisfactory in this case.

4º. The morbid symptoms which they cause are usually intensified by association with bacteria generally of small pathogenic activity: for instance a paratyphus bacillus or a pasteurella plays a role in the pulmonar or intestinal form of hog-cholera (swine-plague); in pox a streptococcus, in the epithelio-thelioma of birds a staphylococcus, and so forth.

5º. The attack the organism suffers, when it does not prove fatal, causes immunity, at times of a very lasting kind, as in pox, epithelioma of birds, yellow fever, etc., at others of very short

term, as in the case ot foot-and-mouth disease.

6o. The animal species, which are sensitive to the germ, produce sera of a very real preventive value: thus serum active against foot-and-mouth disease can only be obtained from cattle and porcines; serum against hog-cholera only from sheep, and so forth.

This last character is one of those which are still rather uncertain in medical and veterinary litterature; it is in this way that, in the case of the contagious epithelioma of birds, the conclusions are categoric and deny the serum any preventive or curative properties. To sustain this last assertion, i. e. "that sensitive species produce a preventive serum", the first indication was to control the experiments of other research-workers on this subject, by repeating them, avoiding causes of error which might have left margin for the obtaining of negative results. That was the reason for the undertaking of this work.

Research work carried out at our Station showed that, here as elsewhere, these errors had been of dual nature; insufficient doses and a pseudo-superimunisation.

A) *Insufficient Doses*. There is a general tendency to carry out the investigation of sera with insufficient doses, a fact which frequently leads to the denying of any value to antibacterian and antitoxic sera of great curative value, such as the one against bubonic plague, tetanus, etc. In the cases of the filterable germs MOUSSU, in preparing serum against foot-and-mouth disease by injections of virulent blood, obtained a serum certainly efficacious, but tried its effect with 50 cm^3 which in this case must surely have corresponded to one third of the efficient dose. On the other hand, when the research-workers use considerable doses the results are positive in foot-and-mouth disease as in diseases produced by other filterable germs, unless another cause of error intervenes.

In vaccination, REYNAUD, among others, was able to immunise calves with 250 cm^3 of a convalescent's serum; and STRAUSS, CHAMBON and MÉNARD, with more or less a litre. (Possibly an intense super-immunisation might reduce this dose to its tenth part; in another publication we will return to the subject). KUNIO SATA ascertained the formation of antibodies in rabbits inoculated either cutaneously or in the cornea.

In sheep-pox, DUCLERC immunised the animals with 190 cm^3 of cured sheep's serum and BORREL was even able to obtain serum of practical use, immunising with a dose of 15 to 20 cm^3.

In foot-and-mouth disease, various research workers verified that the serum of animals, that had recovered from the disease, immunised at the dose of a litre and LOEFFLER, super-immunising cattle, reduced the preventive dose to 100 to 150 cm^3, a dose which can be further reduced to less than 80 cm^3 without concentrating the serum.

In Cattle Plague, a few hundred centimetres of the serum of a convalescent animal are already of use in the prevention of the disease, but KOLLE and TURNER, NICOLLE and ADIL BEY and others, by means of superimmunisation, reduced this dose to about 20 cm^3

In hog-cholera, the serum furnished by superimmunised animals is of the most potent kind since it prevents infection when employed in doses of less than 10 cm^3, but as in the case of all the other sera against filterable microorganisms, its curative properties are practically non-existent, and only demonstrable by the use of enormous doses right in the beginning of the disease.

In chicken pest, JOUAN and STAUB were able to immunise chickens with less than 1 cm^3 of serum obtained from chickens.

In rabies (hydrophobia), BABES was invariably able to protect dogs by means of serum obtained from dogs; MAGALHÃES was able to protect oxen with ox serum (unpublished work carried out at the Experimental Station of Bello Horizonte, Minas Geraes). Homologous sera are, as LOEFFLER and others had already ascertained, always more potent than heterologous ones.

In the case of diseases exclusively affecting man, the verification is more difficult as it entails superimmunising convalescents, however:

In poliomyelitis acuta, FLEXNER and LEWIS, LEVADITI and LANDSTEINER cured monkeys with the sera of convalescents and of superimmunised monkeys; NETTER, GENDRON and TOURRAINE, NOBE'COURT and DARRE', FLEXNER and AMOSS obtained favourable results in patients on whom they employed big doses of the serum of convalescents;

In small-pox, PROWAZEK and ARAGAO indicated the efficacity of twelfth-day serum against the virus; superimmunisation would certainly give more positive results.

In yellow fever, MARCHOUX, SALIMBÉNI and SIMOND ascertained that the serum of convalescents is endowed with undeniable preventive properties and perhaps even with some therapeutic ones.

In scarlet fever, EMIL REISS besides others, obtained good results, even in unfavourable cases, by injecting intravenously 100 cm^3 of convalescent's serum before the fourth days of illness.

B) Pseudo-superimmunisation, i. e., the collecting of serum of patients while they are still ill.

The virus-carriers or rather the persons that eliminate or expel virus *(Virusausscheider)* appear to be ill in all cases, even if the chronic symptoms should be of little account. This fact observed by different authors in the case of foot-and-mouth disease, is easily seen in the case of epithelioma of birds.

A fowl apparently recovered from the illness, nourishing itself plentifully, laying eggs, is considered completely recuperated; on the other hand, if we put it under close observation, we can notice that every now and then it shows a brief shaking of the head. This fowl suffers from a slight chronic coryza, and both the cerebro-spinal fluid and the blood are infectious. In such a case one may think of an *immunitas non sterilisans,* seeing that one obtains an infectious serum.

In order to verify the uncertainties pointed out, several studies were undertaken at the Station. The results obtained with the epithelioma or diphtheria of birds, which offers greater facilities for experimental work may already be published.

When one has to deal with a great number of chicks and fowls infected with epithelioma, one can no longer doubt its identity with the diphtheria of birds.

Not only do all the chicks show more or less marked symptoms of diphtheria in the period to which death ensues, but it is also frequent for diphtheric patches to appear in the course of the development of the epithelioma; besides this it is not altogether uncommon for inoculations to call forth only diphtheria. In fowls subcutaneous or intraperitoneal inoculations of big doses of epithelioma usually provoke diphtheria.

Besides this proof based on inoculation, which had been previously studied by S. VON RATZ and by UHLENHUTH and MANTEUFFEL (1910), the serological proofs which I will publish later, confirm this assertion, although MANTEUFFEL did not obtain any therapeutic results by inoculating 5 cm^3 of the

serum of immune fowls, which is not to be wondered at.

The preparation of anti-epithelioma-tous serum, as it is being carried out, consists in triturating material from epithelioma in a stone mortar, straining it through muslin and filtering it through paper, this being a technic similar to the one we employed in preparing serum against foot-and-mouth disease and other sera which are being worked upon. In the case that was being studied I avoided, in opposition to the one of foot-and-mouth disease, intravenous injections, although I consider them the best for superimmunisation, because in the case of fowls its difficulty renders it unpracticable; I preferred, therefore, intraperitoneal inoculations.

To avoid virus-carriers, I resolved to take only not very young fowls in big lots and to carry out a first test-inoculation on them. Although using birds that by their ages should have had chances of infecting themselves before, I was constantly obliged to set aside more or less 50%, because they would either acquire a marked diphtheria, or else because they would manifest the chronic coryza to which I have referred.

The birds, which in the course of a week show no symptoms of disease, save a slight green diarrhœa, are isolated for superimmunisation. This is done according to the general technic which we adopt in current practice for the preparation of serum against the filterable germs, with the alterations in dose and mode of inoculation required by the case, viz. ;

1rst Inoculation (Test Inoculation) Hypodermic injection of 0,1 gr. of the substance of the skin localisations *(boubas)* triturated and dissolved in physiological saline solution, filtered through muslin and through filter-paper.

2nd Inoculation; in the peritoneum. of 0,5 gr. of the substance of the skin localisations properly triturated, diluted and filtered.

3rd Inoculation; intra-peritoneal inoculation of 1 gr. of the material referred to.

4th Inoculation; intra-peritoneal inoculation of 2 grs. of the material referred to.

5th Inoculation; intra-peritoneal inoculation of 4 grs. of the material referred to.

Superimmunised fowls are killed on the tenth day after the fifth inoculation. The serum should be used the same day, as the preservation of it by means of Carbolic Acid (Phenol) produces in young chicks convulsions sometimes causing death and a great irritation at the site of inoculation. The Assistant-Chemist of the Station, BAETA VIANNA, purposes to obviate this drawback by substituting this chemical substance and besides by concentrating the serum through reduction of volume.

The chicks chosen should be very young in order to avoid the ones that should have become immunised by a previous attack of the disease.

Chicks of more than three months of age are already more resistant to infection and older ones and adults still more so; the latter recover without treatment.

After various tentative experiments I ascertained that a dose of 2,5 cm^3 already protects, when inoculated previously and, at times even when inoculated shortly after the virus.

The following tables are résumés of the more recent experiments:

1rst Series.

Chicks one month of age inoculated with 2,5 cm^3 of antiepitheliomatous serum.

1.–Inj. 5 minutes after virus (rubbed in on the head)..... Did not suffer
2.– « 10 « « « « « « « ..... † (death)
3.– « 15 « « « « « « « ..... †
4.– « 20 « « « « « « « ..... †
5.– « 25 « « « « « « « ..... †
6.–Inoculated only with the material from skin lesions (bouba)..... †

Only the chick injected 5' after the virus did not suffer, all the others died within 33 days. Chicks 4 and 5 had enormous skin localisations *(boubas)* the remainder smaller ones.

2nd Series.

Chicks one month of age inoculated with 3 cm³ of antiepitheliomatous serum and spleen extract.

7.–Inj. 15 minutes before virus (rubbed in on the head)..... Nothing
8.–Id..... Id
9.–Inj. 15 minutes after virus..... Id
10.–Id..... †
11.–Inj. 1 hour after virus..... †
12.–Id..... †
13.–Inj. of spleen extract one hour afterwards..... †
14.–Id..... †
15.–Inoculated only with virus..... †
16.–Id..... †

Chicks having been subjected to injection before the inoculation of virus (rubbed in on the head) did not get infected; even injection 1/4 hour after inoculation would only protect one of the two chicks inoculated; from this point on the protective activity was non-existant. Chicks 11 to 14 only showed diphtheric patches, the remainder skin localisations *(boubas)*.

3rd Series.

Chicks two months old inoculated with 4 cm³ of antiepitheliomatous or normal serum.

17.–Inj. 2 hours before (2 cm³) and 1 hour before (another 2 cm³)..... Did not suffer
18.–Id..... Id
19.–Id with normal chicken serum..... skin localisation (Bouba)
20.–Id. Id..... skin localisation (Bouba)
21.–Inoculated only with virus (rubbed in on the head).. skin localisation (Bouba)

In this experiment also the serum showed an absolute value as a preventive.

Fourth Serice.

Chicks of a little more than two months of age inoculated with 2,4 and cm³. of antiepitheliomatous or normal serum.

Preventive Injections (Antiserum).

22.–Inj. of 2 cm³ 24 hours before virus (hypod. inj.)..... skin localisation (Bouba)
23.–Id..... Did not suffer
24.–Inj. of 2 cm³ 24 and 21 hours before virus..... (Total 4 cm³) Did not suffer

25. – Id. ..... Did not suffer
26. – Id. ..... Did not suffer
27. – Id. ..... Did not suffer
28. – Inj. of 2 cm³ at 24, 21 and 18 hours before virus ..... (Total 6 cm³) Did not suffer
29. – Id ..... Did not suffer
30. – Id. ..... Did not suffer
31. – Id. ..... Did not suffer

Preventive Injections (Normal Serum).

32. – Inj. 2 cm³ of normal serum 24 hours before virus ... skin localisation (Bouba) †
33. – Inj. 2 cm³, 24 and 21 hours before virus (Total 4 cm³) skin localisation (Bouba)
34. – Id ..... skin localisation (Bouba)
35. – Inj. of 2 cm³ 24, 21 and 18 hours before virus (Total 6 cm³) skin local. (Bouba)
36. – Id. ..... skin localisation (Bouba)

Curative Injections (Antiserum).

37. – Inj. 2 cm³ immediately after hypod. inj. of virus ..... Nodules
38. – Id. ..... skin localisation (Bouba)
39. – Inj. 2 cm³ plus 2 cm³ after inj. of virus ..... Nodules
40. – Id. ..... Nodules
41. – Id. ..... Nodules
42. – Id. ..... Nodules
43. – Inj. 2 cm³, plus 2, plus 2 after inj. virus ..... Nodules
44. – Id. ..... Nodules
45. – Id. ..... Nodules
46. – Id. ..... Nodules

Preventive Injections (Normal Serum).

47. – Inj. of 2 cm³ immediately after the inj. of virus ..... skin localisation (Bouba)
48. – Inj. of 2 cm³ plus 2 cm³ after the inj. of virus. (total 4 cm³) skin local. (Bouba) †
49. – Id ..... skin localisation (Bouba) †
50. – Inj. of 2 cm³, plus 2 cm³, plus 2 cm³ after virus ..... skin localisation (Bouba) (killed)
51. – Id. ..... skin localisation (Bouba) †
52. – Id. ..... skin localisation (Bouba)

Controls.

52. – Inoculated only with virus ..... skin localisation (Bouba) †
53. – Id ..... skin localisation (Bouba) †
54. – Id. ..... skin localisation (Bouba)
55. – Id. ..... skin localisation (Bouba)
56. – Id. ..... skin localisation (Bouba)

Chicks do not tolerate injections of more than 2 cm³ to 3 cm³, therefore in this series the injections of 4 to 6 cm³ were made in two or three times at intervals of approximately three hours.

The nodules observed in the abdomen (site of the injection of serum in chicks of numbers 38 to 46) appear to have been due to the carbolic acid needed to preserve the serum; this was not observed in the chicks of lower numbers, injected with fresh serum, nor in the controls treated with normal serum, also in fresh condition.

Here, once more, the preventive va-

lue of the serum was complete, when the serum was used in the dose of 4 to 6 cm^3.

We may therefore conclude that serum against the epithelioma of fowls may be easily obtained provided that;

1º the superimmunised fowls support the inoculations without morbid manifestations, even slight ones (chronic coryza, for instance).

2º the activity of the immunising serum be verified with high doses with regard to the weight of the animal that is to be protected against infection.

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The simple passage of the virulent material through muslin and filter paper might, at first sight, seem to prejudice the experime but I had no intention of demonstrating in any way the filterability of the germ of the diphtheria of fowls; this had already been brilliantly done by great research-workers; my purpose was to show that fowls superimmunised with virulent material furnish serum against a disease produced by a filterable germ that seemed to elude the general principal that; *The filterable germs produce, as a rule, preventive serum when inoculated in a proper manner in animals sensitive to the disease which they provoke.*

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