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ON AQUEOUS HUMOUR, MICRO-ORGANISMS, AND IMMUNITY.

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ON AQUEOUS HUMOUR, MICRO-ORGANISMS, AND IMMUNITY.

By E. METCHNIKOFF.

From the "Institut Pasteur," Paris.



I.

THE aqueous humour of the eye is an organic liquid, containing no cellular elements in the normal state. Hence it presents great interest from the point of view of immunity against infectious disease; for whereas it is impossible to collect blood-plasma, owing to the fact that, when shed, it immediately coagulates, and whereas blood-serum has already undergone grave alterations, it is an easy matter to collect aqueous humour in a perfectly normal state.

Previous investigations have shown that although aqueous humour contains but a small quantity of albuminoid substance, this fluid forms an excellent cultivating medium for micro-organisms in general. Koch's remarkable researches[1] on the bacillus anthracis were based on cultures made in aqueous humour, and even saprophytic micro-organisms, such as bacillus subtilis and bacillus megaterium grow extremely well in the aqueous humour withdrawn from the living body (Trapeznikoff).[2]

But what happens to micro-organisms placed in the aqueous humour of living animals? This problem has been solved by numerous investigations. Ribbert[3] noticed that fungi, which grew only very sparingly in the animal body, developed exceedingly well in the anterior chamber of the eye, or aqueous humour. The bacillus anthracis thrives perfectly in the anterior chamber of the eye, and in the aqueous humour of the eye, of animals naturally immune against anthrax, such as dogs, fowls,[4] or frogs, and also of animals, such as rabbits and sheep, rendered artificially immune against this disease. The same remark holds good for other micro-organisms. Thus the anterior chamber of the eye and aqueous humour of rabbits rendered artificially immune against "rouget" is

[1] Cohn's *Beiträge zur Biologie der Pflanzen*, vol. ii., 1876.
[2] *Annales de l'Institut Pasteur*, 1891, p. 391.
[3] "Untergang der Schimmelpilze im Körper," 1887.
[4] *Annales de l'Institut Pasteur*, 1890, p. 570.

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a good cultivating medium not only for the virulent bacilli, but even for those of the first vaccine of this disease.[1]

Although this fact appears to be generally true, it has nevertheless been controverted by other observers. Gamaleia[2] states that the bacillus anthracis exhibits abnormal shapes only when placed in the aqueous humour of sheep suffering from the fever which follows on the inoculation of anthrax-vaccines. According to this author the bacillus anthracis under such conditions, instead of growing in the normal manner, forms very fine threads, and short, swollen, and degenerated rods.

This question has been re-investigated by O. Metchnikoff,[3] who has proved Gamaleia's statement to be incorrect. This observer has shown that the aqueous humour withdrawn from sheep during the vaccine-fever of anthrax gives abundant and perfectly normal cultures, not only when the strong virus, but even when the two anthrax-vaccines are used for the experiment. The general rule, therefore, instead of being shaken by this so-called exception, is, on the contrary, confirmed by this observation, which shows that the bacillus anthracis grows well in the aqueous humour of sheep during the course of the vaccine-fever of anthrax.

Messrs. Charrin and Roger[4] concluded from their experiments that the bacillus pyocyaneus did not thrive well in the aqueous humour of rabbits rendered artificially immune against the bacillus pyocyaneus, or of those suffering from the pyocyanic disease; and that, under such conditions, the micro-organisms "often present abnormal shapes resembling those found in bacilli growing on media which contain antiseptics."

I have made a large series of experiments in order to verify this assertion, and although these researches have been extremely numerous, and have been repeated time after time during the past two years (1890 and 1891), the results have always been the same—namely, the bacillus pyocyaneus grows as abundantly in the aqueous humour of immune rabbits as in that of non-immune animals, and, in all, the bacilli which develop are small and perfectly normal. The tendency to form beaded chains, which, according to Messrs. Charrin and Roger may be noticed in bacilli growing in the serum of immune rabbits, cannot be observed in the aqueous humour of the same animals.

This second so-called exception therefore has no surer basis than the first, so that the general rule already mentioned is fully confirmed. Several new examples supporting it may now be quoted.

The aqueous humour of rabbits rendered immune against the

[1] *Annales de l'Institut Pasteur*, 1888.

[2] *Annales de l'Institut Pasteur*, 1888, p. 544.

[3] *Annales de l'Institut Pasteur*, 1891, p. 150.

[4] *Comptes Rendus de l'Académie des Sciences*, 1889, No. 19, p. 713. Although Messrs. Charrin and Roger speak of their experiments as being "incomplete," yet they have not rectified their mistake in any of their subsequent publications on the bactericidal properties of animal fluids.

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pneumococcus of Fraenkel is a very favourable cultivating medium for this microbe. Several experiments with the aqueous humour of rabbits rendered immune by the soluble poisons secreted by the pneumococcus, or by the injection of living pneumococci have shown that, in all cases, this micro-organism grows abundantly when inoculated into the aqueous humour of such animals, forming micro- or diplo-cocci, and occasionally, very short, beaded chains containing not more than four segments. Frequently the pneumococcus grows even better in the aqueous humour of immune than of normal rabbits, and renders the liquid opaque without giving rise to any considerable deposit.

The micro-organism of "hog cholera" also finds an excellent cultivating medium in the aqueous humour of rabbits rendered immune against this disease; the microbes cultivated under such conditions develop, quite normally, into oval bacteria and characteristic diplococci.

The same remarks apply to the "vibrio Metchnikovii" when this is cultivated in the aqueous humour of guinea-pigs rendered immune against the septicæmia produced by this microbe.[1]

The general conclusion to be drawn, therefore, is,—that micro-organisms develop abundantly and quite normally in the aqueous humour of naturally or artificially immune animals.

II.

In order to supplement the facts related in the first part of this paper, it is necessary to give an account of the bactericidal property of the aqueous humour of various animals.

Nuttall[2] was the first to prove that the aqueous humour of rabbits has a bactericidal action on the bacillus anthracis. Buchner[3] has confirmed this result, and has made similar observations on the aqueous humour of dogs and rabbits with regard to the bacillus of typhoid fever.

How, then, is this well-established fact to be reconciled with the general rule that the aqueous humour forms an excellent cultivating medium for micro-organisms?

In the usual experiments on the bactericidal properties of animal fluids, the organic fluids are simply collected into test-tubes and inoculated with small quantities of micro-organisms, which, in the large majority of cases, have been growing on totally different media. Now, the sudden transfer from one medium to another very often proves fatal to a large number of micro-organisms. If, on the contrary, care be taken gradually to accustom a given microbe to media derived from the animal organism, it is possible to cultivate this microbe exceedingly well

[1] *Annales de l'Institut Pasteur*, 1891, p. 473.
[2] *Zeitschrift für Hygiene*, vol. iv., 1888, p. 382.
[3] *Arch. f. Hygiene*, vol. x., 1889, p. 151.

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in such liquids. Thus, Hafkine,[1] in a series of investigations carried out in my laboratory, has obtained very abundant colonies of the bacillus of typhoid fever in the aqueous humour, although at the beginning of his experiments, this liquid proved to have marked bactericidal properties.

If the aqueous humour be tested in the usual manner, it is found to exert bactericidal action on certain kinds of micro-organisms—the bacillus anthracis, for instance—and it matters not whether the aqueous humour be derived from animals non-immune or immune against anthrax. The following experiment proves the correctness of this statement.

The aqueous humour of a rabbit which had been rendered immune against anthrax, and that of a normal rabbit, possessing no resisting power against that disease, were separately inoculated with the contents of one platinum loop which had been dipped into the blood of a rabbit dead of anthrax. Immediately afterwards gelatine plates (Petri's) were prepared and inoculated with one platinum loop of aqueous humour in which the anthrax bacilli had been sown. The same operation was repeated 5 minutes, 2½ hours, and 5 hours after the beginning of the experiment, and the examination of the plates gave the following results:—

	Aqueous Humour of Immune Rabbit.	Aqueous Humour of Normal Rabbit.
Number of anthrax colonies immediately after inoculation, ...	107	184
Five minutes after, ...	0	12
Two hours and a half after, . . .	0	0
Five hours after, ...	0	0

All the bacilli perished a very short time after inoculation, in the aqueous humour of the immune and non-immune rabbit alike.

Such a result, however, is only obtained when a very small number of bacilli is inoculated; when a larger number is introduced, the bacilli adapt themselves more easily, some are able to live in the new medium, and finally give an abundant culture. Thus, in another experiment with the aqueous humour of an immune and of a normal rabbit, the result was quite different, the number of colonies on the gelatine plates being as follows:—

	Immune Rabbit.	Normal Rabbit.
Number of colonies immediately after inoculation of bacilli into the aqueous humour, ...	2624	1755
Forty minutes after, ...	2583	726
Three hours after, ...	1512	4000

The aqueous humour of both rabbits at first caused the number of bacilli to diminish; but instead of their all perishing, as in the first

[1] *Annales de l'Institut Pasteur*, 1890, p. 377.

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experiment, some survived and ultimately gave rise to an abundant new culture.

On comparing these two columns of figures it might be concluded that the bacilli adapt themselves more easily to the aqueous humour of the normal, than to that of the immune rabbit. This particular result, however, was due merely to chance, for I noted in several experiments that, whereas the bacilli died in the aqueous humour of non-immune animals, they survived in that of rabbits rendered artificially immune. In one experiment I compared the aqueous humour of a guinea-pig—an animal far more subject to anthrax even than a rabbit—with the aqueous humour of a rabbit rendered artificially immune. The inoculation of an extremely small quantity of bacilli gave the following results:—

	Aqueous Humour of Guinea Pig.	Aqueous Humour of Rabbit.
Number of colonies immediately after inoculation, ...	5	11
Thirty-three minutes after, . .	0	0
Three hours after, ...	0	11

In this experiment the aqueous humour of the guinea-pig was proved to possess far greater bactericidal power than that of the immune rabbit.

There is no relation, therefore, between the bactericidal powers of aqueous humour and immunity. In fact this conclusion is self-evident, for it has already been proved by those who have previously studied the question, that the aqueous humour of non-immune rabbits has a marked bactericidal power on the anthrax bacillus.

The contradiction between the existence of this bactericidal power *in vitro*, and the fact that the animals yielding this aqueous humour are extremely sensitive to anthrax, is but an apparent one.[1] As a rule, when a drop of fluid containing anthrax bacilli is introduced into a tube containing aqueous humour, the observer at once shakes the tube and scatters the micro-organisms, so that all the microbes are immediately acted upon by the aqueous humour; but if the same number of bacilli be introduced into the anterior chamber of the eye, the microbes remain near each other for some time, and hence are subjected far more slowly to the influence of the aqueous humour. Now, this last condition is a most important factor in enabling the bacilli to adapt themselves to existence in a new medium.

If in making cultures of bacilli in aqueous humour, one drop containing the micro-organisms is introduced and is not distributed throughout the fluid, as occurs where plates are to be made, the growth of the organism continues. This is the reason that simple inoculation nearly always gives abundant cultures, whereas inoculation followed by the making of plates often gives negative results.

[1] *Virchow's Archiv*, 1888, vol. cxiv., p. 472.

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III.

Having now proved that the aqueous humour of immune and non-immune animals is a very favourable cultivating medium for micro-organisms, and that the form of such organisms grown in this fluid is perfectly normal, we may now proceed to study the functional modifications of bacilli cultivated in this liquid.

The problem to be solved is, Whether the bacilli which grow so well in the aqueous humour of immune animals are able to produce colouring matters, and more especially toxines under such conditions? I particularly mention colouring matters, because MM. Charrin and Roger frequently lay special stress on the fact that the bacillus pyocyaneus, when cultivated on media derived from animals rendered immune against the pyocyanic disease, secretes scarcely a trace of pyocyanin.

I used for my experiments a variety of bacillus pyocyaneus, which secreted a large quantity of green, fluorescent material, but which had lost the property of secreting pyocyanin owing to the fact that it had passed a great many times through the animal organism.[1] Now, the bacilli cultivated in the aqueous humour of immune rabbits produced quite as much green colouring matter as those grown in the same liquid withdrawn from non-immune animals. In the course of my numerous experiments, I came across only one fact requiring special mention, which is, that the green substance is secreted in quantities far larger than usual when the bacillus pyocyaneus grows in the aqueous humour of rabbits suffering from an acute attack of the disease. The reason for this is, that the aqueous humour of such animals, instead of remaining liquid, coagulates almost instantaneously after having been withdrawn from the eye.

The question as to whether the toxine-producing capacity has undergone any change is of far greater importance. What is the virulence of micro-organisms growing in the aqueous humour of immune rabbits? Does the virulence become less in such cases, owing to the presence of that property which has usually been described as the "attenuating property" of animal fluids?

My investigations were made with five kinds of micro-organisms, namely, the bacillus pyocyaneus, the pneumococcus of Fraenkel, the bacillus anthracis, the micrococcus of hog-cholera, and the vibrio Metchnikovii. These experiments showed that the first four classes of micro-organisms, when cultivated in the aqueous humour of rabbits rendered artificially immune, and the vibrio Metchnikovii cultivated in the aqueous humour of guinea-pigs rendered immune, showed no diminution in their pathogenic effects on rabbits and guinea-pigs respectively.

In my whole series of researches, I met with only one case in which the cultures of a micro-organism in the aqueous humour of an animal

[1] See the paper by Gessard, *Annales de l'Institut Pasteur*, 1891, p. 65.

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rendered artificially immune, did not produce death. This exception occurred in the case of pneumococci cultivated in the aqueous humour of a rabbit which Dr. Roux had rendered immune against the pneumococcus of Fraenkel; a rabbit inoculated with this culture recovered, whereas the control animal, inoculated with the pneumococcus cultivated in the aqueous humour of a normal rabbit, died in thirty-six hours. In four other experiments, however, the rabbits inoculated with cultures of pneumococci in the aqueous humour of immune rabbits, died at nearly the same time as the control animals, and in one case, even before the control animal.

On the other hand, it happened twice in my experiments with the bacillus pyocyaneus that the control animals recovered, whilst the rabbits inoculated with cultures made in the aqueous humour of immune rabbits died several days after their inoculation. This result cannot be attributed to an augmentation of virulence in the bacilli growing in the aqueous humour of rabbits rendered artificially immune, but is rather to be explained by the fact that the quantity of aqueous humour to be obtained from the eye is never sufficiently large, and that in order to give the pyocyanic disease to animals, small or medium doses are generally not sufficient. This deficiency in the quantity of aqueous humour is also a drawback to the experiments made with the vibrio Metchnikovii, when this organism is cultivated in the aqueous humour of guinea-pigs rendered artificially immune. Even if one withdraws this liquid from both eyes, the quantity obtained is inadequate to carry out experiments with adult guinea-pigs. But if young animals are used,[1] it is easy to see that the vibrio cultivated in the aqueous humour of immune guinea-pigs proves quite as deadly as the cultures made in the same liquid obtained from normal, non-immune animals.

On this account, experiments with anthrax or hog-cholera are far more satisfactory, as infinitesimal quantities of these pathogenic microbes are sufficient to kill inoculated animals with certainty. In one experiment made on January 19th, 1891, two drops of a culture of the bacillus made in the aqueous humour of a rabbit rendered immune against anthrax, killed another rabbit in $53\frac{1}{4}$ hours; this second rabbit exhibited all the classical signs of anthrax.

In another experiment made on January 24th, 1892, I used a culture of the microbe of hog-cholera grown in the aqueous humour of a rabbit rendered immune against that disease. A few drops of this culture, injected into the auricular vein of a rabbit weighing 2013 grammes, proved fatal in ten hours, the blood of the heart being found to contain a number of the micro-organisms of hog-cholera. The control rabbits inoculated, in the same manner, with a culture made in the aqueous humour of a non-immune rabbit weighing 1995 grammes, died several hours later than the first animal.

The so-called "attenuating property," therefore, is never met with

[1] Where a smaller quantity is sufficient to produce a lethal effect.—(ED.)

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in the aqueous humour of animals rendered artificially immune, the cultures made in such media always proving very virulent.

Is it possible that the aqueous humour possesses the so-called toxinicidal property which has been claimed by Behring and Kitasato[1] for the serum of rabbits rendered immune against tetanus? According to Vaillard's[2] researches, the aqueous humour of immune rabbits in no wise impairs the toxic action of tetanus. Investigations into the toxinicidal property of aqueous humour for other diseases are not, as yet, feasible, owing to our deficient knowledge of this property in general.

The results of these investigations may therefore be summed up in the following conclusions:—

1. That aqueous humour is a good cultivating medium, not only for micro-organisms in general, but also for that special microbe to which the animal from which the aqueous humour has been taken is immune.

2. In a few cases the bactericidal property of aqueous humour may be demonstrated, but in no case does this property stand in any relation to the immunity of the animal furnishing this fluid.

3. The micro-organisms cultivated in the aqueous humour of animals rendered artificially immune retain their normal virulence.

4. The so-called toxinicidal property can, in no wise, be attributed to the aqueous humour of animals rendered artificially immune.

[1] *Deutsche Med. Wochenschrift*, 1890, p. 1113.
[2] *Comptes Rendus de la Soc. de Biologie*, 1891, p. 463.



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