Bacteriology. — "*On the so-called filtrable virus of influenza described by* VON ANGERER." By L. K. WOLFF. (Communicated by Prof. C. EYKMAN).

(Communicated in the meeting of June 28, 1919).

Towards the end of 1918 VON ANGERER [1]) published communications on a virus of influenza, discovered by him. He injected rats with sputa of sufferers from influenza, filtered the blood of these rats germfree, when they were already very ill and put the filtrate into glucose-broth. After incubation at a temperature of 37° C. this broth became turbid, without bacteria distinctly being found in them. Yet VON ANGERER describes very small formations, angioplasmata, which he considers the cause of the influenza. This communication was confirmed and completed by himself and other investigators [2]). The result of these researches was that the rat was no longer necessary for isolating the virus, but that it was sufficient to add blood of sufferers from influenza to the broth.

While investigating, together with DR. SNAPPER [3]) the secondary bacteria that are the cause of pneumonia in influenza-patients, we have sometimes observed this turbidness, without being able to find any microbe in the liquid.

Yet we were struck by the fact, that a great number of round, gram-negative granules were to be found in such a broth, but the unequal size had prevented us from considering these formations as bacteria. After the communications of VON ANGERER had been published, I have paid more attention to this turbidness, which is obtained by inoculating the blood of influenza-patients into glucose-broth and by incubating this liquid at 37° C., and I have been able to observe them in three cases of serious influenza-pneumonia. I must add at once however that I found them also in a case of endo-

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[1]) Münchener Med. Woch. 1918, N°. 46 and 47.

[2]) PRELL: ibidem 1918, N°. 52.

LESCHKE, Berlin. Klin. Woch. 1919, N°. 1.

See further OLSEN (Report Aertzl. Verein Hamburg Jan. 7th 1919) and KRONBERGER, Deutsche Med. Woch 1919, N°. 9, who consider the results of VON ANGERER non-specific.

[3]) Tijdschr. v. Geneesk. 1919, p. 1483.

carditis lenta in a child, where I did not find streptococci in the blood.

The epidemic had nearly reached its end, and I should not have been able to continue my researches if not a happy coincidence, a wrong hypothesis, as appeared afterwards, had helped me on.

Starting from the fact, observed by myself and also by other investigators, that inoculation of dead bacteria, which complicate the influenza, so pneumo- and streptococci or influenza-bacilli, on persons not only protects them against complications, but also against the influenza itself, I thought that the virus of the influenza would probably be present in the cultures in broth with blood of strepto- and pneumococci, collected by Dr. SNAPPER and myself, and so I tried to separate the virus by filtration through a Berkefeld-filter and inoculation into broth with blood. It actually succeeded the first times. I obtained liquids in which no ordinary bacteria were present, but which became turbid at 37° C. My results were however varying, at one time the liquid became turbid, at another time it did not.

After first having ascribed these varying results to the Berkefeld filters, it became evident afterwards that the presence or the absence of the turbidness was dependent on an addition of a small quantity of hemoglobin and now the riddle was soon solved. If one adds to the broth a liquid containing a small quantity of hemoglobin, this mixture remains clear at room-temperature, but it becomes turbid in the incubator after 24 hours. This turbidness is also formed in peptone, even in salt solution; the latter must be however very precisely neutral, because otherwise the turbidness is not observed. The hemoglobin solution was always made by washing erythrocytes with salt solution, then dissolving them in distilled water and filtering through a Berkefeld-filter. It is easy to give an explanation, why this turbidness is obtained in blood from serious influenza patients; in this illness a slight hemolysis of the blood arises intra vitam through the secondary hemolytic streptococci, and the blood we add to the broth will contain not only red blood corpuscles, but also hemoglobin, free in the plasma. And this is broken up in the incubator.

To prove this more closely I prepared the carotis of a rabbit free, let a few drops of blood flow into the broth and into a test tube (I). Then I injected distilled water in the earvein and shortly afterwards blood was drawn from the carotis and mixed in the broth and in a test tube (II). The tubes with broth were put in an incubator. I let the blood, which was received in the testtubes, coagulate;

the second contained pale red serum and spectroscopic oxyhemoglobin. After 24 hours the broth in the test tube II was decidedly turbid after slight centrifuging to remove the erythrocytes, the broth in the test-tube I was clear. Both proved to be sterile.

The question has still to be answered, what may be the cause of the turbidness. If one adds a little more hemoglobin-solution to the broth and leaves it in the incubator, the next day a turbid liquid and a red precipitate are obtained; the latter does not dissolve, or only with great difficulty in acids, but easily in diluted alkalies. The solution does not show absorptionbands in the spectroscope; in adding a little ammonium sulphide, we get directly a distinct band, characteristic of hemochromogen. If we first add potassium cyanide, and then ammonium sulphide, we get two bands, of which the left one has moved a little towards the red in comparison with the above band. All this points to the fact that we were dealing with hematin

It is obvious that we have an autolysis of the hemoglobin. In most cases the globin will remain dissolved, as the broth is not exactly neutral; in neutral salt solution it may add however to the turbidness. If one wants to obtain the turbidness of hemoglobin in salt solution, one ought to take highly diluted hemoglobin solutions, otherwise it does not appear. This happens, because the reaction of the salt solution changes by adding a great quantity of hemoglobin solution.

The fact of getting turbid at 37° C. of tubes of broth and blood that has been drawn from the body a considerable time ago, a well-kown fact to those, who experiment with this cultureliquid, depends of course on the same fact: autolysis and the formation of hematin.

Recapitulating the facts, we may say that the turbidness of broth, described by VON ANGERER after adding the filtrate of the blood of serious influenza patients, is not specific, but must appear everywhere, where in the blood an important destruction of erythrocytes has taken place. The turbidness is not a virus, but hematin (and globin) originating from the hemoglobin present.

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