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Vaccination and Chemo-vaccinotherapy of Q fever in White Mice.

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AccorAccording to data of numerous researchers (Krasinskaya, 1952; Seigert 1950, 1953; Brogle, 1951; Saikos, 1951; Fisher, 1952) who have worked with rickettsiosis and other infections, the early application of antibodies, being the most effective, leads to a weakening of the development of immunity. Analogical results were obtained by us during study of experimental vesicular rickettsiosis in white Mice. In such a case, a stimulating action on the development of immunity can be rendered by the application of antibiotics in combination with vaccine (Planeles, Krasinskaya).

The task of this study was the experimental development(in tests on mice) of the most rational combined application of antibiotics and vaccine during Q fever.

In so far as a necessary phase of the study of a combined application of antibiotics and vaccine is the study of the postvaccinal immunity, we started the study with the development of this very problem.

The method of testing was composed of the following. Mice weighing 12-13 grams were vaccinated subcutaneously with ordinary and precipitated, killed vaccine of various concentrations- with a content of 259 and 900 million microbe bodies in 1 ml (according to bacterial standards). The vaccine (Vasiliva) was prepared from the Grit strain of the Bernet rickettsia, this being cultured on chicken embryo. The ordinary vaccine was injected thrice with intervals of 7 days, in the following doses: 0.25, 0,25 and 0.5 ml; the precipitated vaccine was injected twice with an

interval of 14 days in equal doses of 0.5 ml. The presence of immunity was determined by means of intravenous infection of mice with a standard dry culture of Bernet rickettsia in dilutions corresponding to the dilution of the yolk sac 1:20.

We had to utilize such large doses of infecting material because, the status of immunity was evaluated according to the presence or absence of rickettsia in the imprints of spleen on the 3rd, 5th and 9th day after infection, and microscopic detection of rickettsia in grown mice, weighing 22-24 grams, in our tests was possible only during massive intrevenour injections.

Controls in these tests were non-vaccinated mice of a heavy weight (22-24 grams), and mice which had recovered from Q fever, infected with this same size dose.

On the 3rd, 5th and 9th day after infection, in control animals not previously ill, there was observed a sharp enlargment of the spleen and an abundant accumulation of rickettsia in it, in the form of micro-colonies; in the recovered mice, re-infected after 40-55 days, the rickettsia in the first 3 days after the secondary infection were subjected to lysis (microscopy of imprints of spleen), and in the following days were not detected microscopically. However, the spleen greatly increased in size after the second infection, in comparison with the spleen of mice infected for the first time, with dissection after 40-55 days. The results of the testing of immunity after vaccination are on Table 1.

Immunization with the ordinary vaccine (during testing of immunity with large doses) showed no good effect—rickettsia in the imprints of spleen at the height of infection were detected in large quantities, even though in the first days there was observed a partial lysis of them. In

the reverse, immunization with a precipitated vaccine proved quite effective--rickettsia by the 5th day was no longer microscopically detectable. However, a full cleansing of the organism from rickettsia, as was indicated by bio-assays on guinea pigs, even during this was not accomplished.

Because the precipitated vaccine caused the formation of stable infiltrates at the locale of injection, in further tests we utilized only the ordinary vaccine.

The next phase of work was the study of the effect of chemio-therapy on the course of infection in the vaccinated organism. In the beginning the tests were run in those conditions when neither vaccination nor treatment alone proved effective. To accomplish this, the vaccinated mice were infected intravenously with a dry egg culture of Bernet rickettsia (0.2 ml of yolk sac diluted 1:20) 52 days after the last inoculation and were at once treated with biomycin for 4 days with a lower than minimum effective dose--0.6 mg per mouse in one day (minimum effective dose equal to 1 mg per day). Treatment of the non-vaccinated mice with such a dose of biomycin proved in-effective--rickettsia was detected in the spleen. The results of the treatment of vaccinated mice are set in Table 2, from which is visible that the biomycin, applied in a dose lower then minimumly effective, in a vaccinated organism proved quite effective, causing lysis of the rickettsia. Irregardless of the visible lysis of rickettsia, the latter remains in the organism, this was proven by positive readings of bio-tests on guinea pigs 9 days after the test.

Further on we were interested in whether or not a preliminary vaccination aids in the sterilization of the organism during treatment with large doses of biomycin. For this we infected vaccinated mice with two

different doses of egg culture (dilutions 1:50 and 1:1000) of the Bernet rickettsia (yolk sac) and treated them with biomycin in doses 5 times higher than the minimum effective, that is, 5 mg per mouse. Under these conditions the spleen was not enlarged nor was rickettsia microscopically detectable.

We judged the retainment of rickettsia in an organism according to the results of biotests on chicken embryo. For this a sterilly taken spleen was pulverized by beads in a container, diluted in 5 ml of a physiological solution and the obtained suspension was injected into the membrane of the yolk sac of a 7 day chick embryo. In case of absence of rickettsia in the smear from the yolk sac, 'blind passages' were conducted.. Usually one or two passages were sufficient for the appearence of rickettsia. In one series of tests we dissected the mice after two days, immediately after treatment and after a month. Results of bio-assays indicated that even during treatment with large dose of biomycin the sterilization of the organism is not completed, as in the preliminarily vaccinated and treated mice, so in mice treated only, and during infection with both doses principally the same results were obtained. A patho-morphological study disclosed a difference in the reaction of the vaccinated and non-vaccinated animals. Thus, in the infected and treated mice there were observed small hyperplasia and swelling of the reticular cells of the spleen, in the liver--analogical variations of the Kupffer cells. Degeneration was fully absent. These variations sharply varied from those variations which were observed in the control animals, but all the same they indicate the presence of a reaction to the introduction of the infecting material. In the organs of vaccinated and treated mice the oppisite, the characteristic variations

were absent, which, evidently, indicate a more absolute protection of the organism against infection. It is necessary to note that in all cases, upon the introduction of large doses of biomycin, the liver reacted with appearences of degenerative steatosis which was most expressed in the first days after treatment and decreased by the third week.

Thus, the preliminary vaccination of the mice meant that the infection in the vaccinated organism is more readily subjected to the action of the antibiotics, but the agent still maintains itself in the organism.

In the last series of tests the vaccination was combined immediately with the infection and treatment. The scheme of the combined vaccination was as follows: the first injection of vaccine (150 million microbic bodies) was on the day of infection, the second (50 million)- on the 5th day, in this same period the treatment with large doses of biomycin was started and lasted 4 days; after the end of the treatment the last injection of vaccine (50 million) was completed. The control mice were only infected or infected and treated, but not vaccinated. Dissection of the animals was 30 or 56 days after infection.

Results of the bio-assays on guinea pigs indicated that after one month the rickettsia was still present in the control animals as well as in the treated, but after 2 months the rickettsia was not detected in the vaccinated and treated mice while in the non-vaccinated animals the bio-assays remained positive.

Conclusions

1. The combination of a preliminary vaccination with treatment with biomycin strenghtened the action of the latter, which is evident with small doses of the preparation.

2. Irregerdless of the good treating effect of the combination of a preliminary vaccination and a later treatment, rickettsia remains in the organism.

3. A combination of vaccination directly with infection and treatment aids in a quicker cleansing of the agent from the organism.

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Table 1. Vaccination of mice against Q Fever

Vaccine	Concentration of vaccine in millions	Number of injections and dosage in Milliliter	Day of test of immunity	Dissection of Mice at high point of infection..... 1/	Content of rickettsia in spleen
Ordinary	250	0.25-0.5--0.5	28th	9/9	++, +++
	250	" " "	43rd	8/8	++, +++
	250	" " "	52nd	5/6	++
	900	" " "	52nd	4/6	++, +++
Precipitated(deposited)	250	0.5-0.5	52nd	0/6	
	900	0.5-0.5	43rd	0/9	
	900	0.5-0.5	52nd	0/6	
Control	—	—	—	8/8	+++

1/ Numerater--Mice containing rickettsia; Denominator--Mice dissected.

This same footnote applies to Table 2.

Table 2. Treatment of mice vaccinated against Q-fever.

						Mg of biomycin....
Ordinary	900	0.25-0.5--0.5	52nd	4/6	++/+++	--
	900	"	52nd	0.6		0.6
	250	"	52nd	5/6	++	—
	250	"	52nd	1/6	Tolerance unit	0.6
Non-vaccinated	—	—	—	5/6	+/++	0.6
Control	—	—	—	6/16	+++/++++	—