

EFFECT OF CERTAIN ANTIBIOTICS ON ACTIVE IMMUNITY IN WHITE MICE

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The question of the influence of antibiotics on the immunological processes of the body acquires a greater significance in connection with the wide use of these preparations in the treatment of a whole series of infectious diseases.

The extensive literature devoted to the study of the influence of antibiotics on the production of antibodies hardly touches on the question of the effect of antibiotics on the survival of vaccinated animals infected with a virulent strain of a pathogen infectious for them.

Having established in previous work [1, 3] that prolonged administration of a number of antibiotics acts unfavorably on the production and circulation in the blood of agglutinins, we decided to investigate whether this demonstrates an influence of these substances on the production of active immunity in white mice. With this aim in mind we selected penicillin, streptomycin, syntomycin and its levo- and dextrorotatory components, levomycetin and dextromycetin.

EXPERIMENTAL METHODS

We divided mice weighing 18-20 g into 3 groups in each experiment. The mice of the first (experimental) group were vaccinated by three injections of killed typhoid vaccine, obtained from the N. F. Gamaleya Institute, in doses of 100, 200, and 200 million microbes at intervals of 7 days. The mice also received at the same time as the vaccine one of the antibiotics being studied.

The mice of the second (control) group were vaccinated only.

To the third (control) group belonged the mice receiving only the antibiotic being investigated (without vaccination). This control group was designed to reveal whether merely the injection of the antibiotic might produce some effect on the survival of the mice after infection.

We administered the syntomycin, levomycetin and dextromycetin to mice by mouth in 0.5 ml of a 1% starch solution from a special dispenser, twice a day, daily, in the amount of 4 mg of the material per mouse.

The vaccinated control mice received only pure starch. We administered the penicillin and streptomycin in distilled water in the amount of 1000 units per mouse. At the end of the course of vaccination the mice received the preparation under investigation in the course of a further 10 days, but 2 days before infection the administration of the antibiotics was stopped, and the mice infected by intraperitoneal injection of typhoid culture.

In order to make sure that at the time of infection the bodies of the mice did not contain antibiotics in concentrations sufficient to protect them from the action of the injected microbes, we determined the presence of these substances in the liver and blood serum of the mice.

TABLE 1

Effect of Antibiotics on Microbial Growth

Antibiotic	Standard																			
	concentrations (in gamma)										Liver									
	2100'0	2200'0	910'0	210'0	0.35	0.07	0.15	0.31	0.62	1.25	2.5	5	10	12	11	10	9	8	7	6
Syntomycin	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
Levomycetin	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
Dextromycin	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
Penicillin	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
Streptomycin	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++

We ground up the liver in a sterile mortar with quartz sand and physiological saline in the proportions of 1 ml saline to 1 g liver. We centrifuged the emulsion, transferred 0.2 ml of the supernatant fluid to a test tube containing 0.2 ml of Hiss' medium with glucose infected with the test organism (*Staphylococcus* in titrating penicillin and enteric bacilli in titrating streptomycin and syntomycin). We inoculated the Hiss medium by adding 250,000 bacilli to 1 ml. We carried out the titrations by the method of serial dilutions.

Serum of blood taken from the mice by cardiac puncture was titrated for the property mentioned above. As a control we took the antibiotic under investigation in the amount of 10 γ of the substance for the initial dilution. In all 12 dilutions were made, starting with a 1:2. We placed the test tubes in the thermostat for 18 hours, then determined the presence or absence of the antibiotic by the change in color of the Hiss medium which attested growth of the microbes.

EXPERIMENTAL RESULTS

As is seen from Table 1, about 24 hours after completion of the administration of the antibiotics they are already not demonstrable in the liver and serum.

Thus we were able to infect the experimental and control animals without fear that the chemotherapeutic preparation was still circulating in their bodies.

The principal requirement, without which it would not be possible to set up experiments to detect active immunity, is the availability of a pathogen, in particular typhoid, of a strain of sufficiently high virulence. But even when infecting with strains of high virulence, the numbers of microorganisms required to bring about the death of the experimental animal sometimes reach very high figures (hundreds of millions). It must accordingly be admitted that the death of mice, when such colossal doses of culture are employed, depends to a significant degree upon the intoxication produced by the typhoid endotoxin which is liberated when the microorganisms disintegrate [6].

This fact explains the desire to increase the virulence of the microorganisms so as to reduce as much as possible the amount of them introduced.

In our method of infecting mice we used a young broth culture. We used a 4-hour broth culture, since according to the findings of Craigie and Brandon [4] Khristova-Baldina [2], and others this culture contains the largest proportion of V-forms, i.e., forms with the high virulence; in addition, we added to the broth 0.5% glucose, which stimulated the development of the bacteria and promoted the accumulation of the Vi antigen [5].

TABLE 2

Survival of Mice Following Treatment with Antibiotics

Group of animals	Number of mice in experiment	Number of mice surviving (actual numbers)
1. Experiment		
Vaccinated mice receiving antibiotic		
Syntomycin	100	50
Levomycetin	100	70
Dextromy	90	9
Penicillin	90	9
Streptomycin	80	16
2. Experiment		
Vaccinated mice	100	70
3. Control		
Mice receiving only		
Syntomycin	25	0
Levomycetin	25	1
Dextromycetin	25	0
Penicillin	23	0
Streptomycin	25	0

To infect the mice in a conclusive experiment we employed strain 4446, the MLD of which was 0.125 ml of a 4-hour broth culture, which contained approximately 120 million microorganisms. After infection the mice were kept under observation for 5 days.

In the results of the experiments we carried out, the following was obtained (Table 2). Mice which had received only antibiotic succumbed after infection in 10% of the cases during the 5 day period; i.e., none of the antibiotics used showed any protective action against the infection which followed. Thirty percent of the control mice, merely vaccinated with typhoid vaccine and not receiving antibiotics, succumbed; the same percent of the mice receiving levomycetin succumbed, as of the controls, but with the difference that death occurred somewhat earlier in this group, i.e., the mice died after about 24 hours, while the controls died in the course of 3 days.

In the experiment using syntomycin 50% of the mice died, and in the group receiving dextromycin, 90%.

Thus on the basis of these data it may be suggested that prolonged application of levomycetin does not exert any important influence on the building up of immunity in white mice.

The application of syntomycin, and especially dextromycetin, hinders the development of immunity. These results make possible the suggestion that the negative influence of syntomycin depends on its levorotatory inactive component. In experiments in which penicillin was used 90% of the animals succumbed, and in experiments in which streptomycin was used, 80%, which clearly demonstrates the inhibitory action of these preparations on immunogenesis in experimental animals.

SUMMARY

Certain antibiotics exert a negative influence on the production of active immunity by white mice immunized with killed typhoid vaccine. During the course of immunization various antibiotics were administered and mortalities of the various groups of mice, following experimental infection, recorded. If syntomycin were given 50% of the mice died, if dextromycin, 90%, penicillin, 90%, streptomycin 80%; 30% of the control group

and the group receiving levomycetin died. However the latter succumbed in 24 hours, while those in the control group died after 3 days.

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