DOSE – EFFECT RELATIONSHIP FOR IMMUNIZATION
OF MICE WITH PURIFIED OEDEMATIENS TOXOID

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The results of a study of the dose – effect relationship for immunization with purified oedematiens toxoid are described. Within the dose range studied (from 1 to 50 antigen units) this relationship obeys the equation of antigenicity. The parameters of this equation were determined for the case under examination.

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Many investigations have been made of the principles governing the immunologic response in relation to dose of toxoid [1-3], but in most of them the preparations used were insufficiently purified. It is quite clear that the accompanying ballast proteins may have modified the true picture of the immunologic response to the antigen concerned because of possible effects of stimulation or competition.

In the present investigation the dose – effect relationships were studied in mice immunized with highly purified oedematiens toxoid obtained by ourselves in 1967.

EXPERIMENTAL METHOD

The oedematiens toxoid used had a specific activity of 3900 antigen units/mg protein nitrogen and was kept in a lyophilized state. The toxoid was dissolved and adsorbed on $Al(OH)_3$.

Noninbred albino mice were immunized subcutaneously with doses of 1, 2, 5, 10, 20, and 50 antigen units. The dose of adsorbent was the same for all doses. In the two experiments 575 mice were immunized. On the 20th day after the single immunizing dose the mice received intramuscular injections of various doses of oedematiens toxin because this method of administration is associated with maximal sensitivity of the animals to the toxin [4-6].

The value of LD_{50} of oedematiens toxin was determined for each group of immunized mice. Bliss's probit method [6] was used to calculate the values of LD_{50}.

EXPERIMENTAL RESULTS

Dose – effect graphs for each experiment separately and a graph of the equation of antigenicity plotted on the basis of the mean results of both experiments are given in Fig. 1. They show that in the dose range from 1 to 50 antigen units the level of immunity, expressed as log LD_{50}, is a linear function of the logarithm of the immunizing dose, i.e., that within the dose range studied, the dose – effect relationship obeys the equation of antigenicity which, in this particular case, has the form:
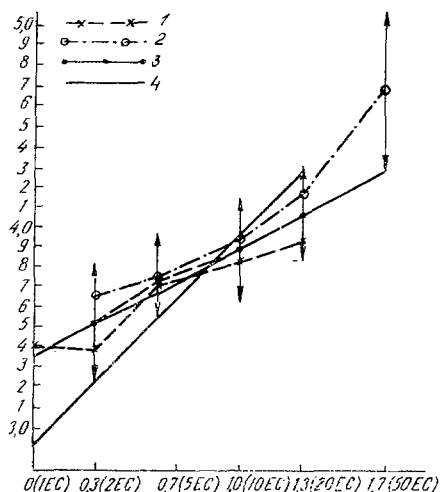

Fig. 1. Dose-effect relationship for immunization with purified oedematiens toxoid. 1) Experiment 1; II) experiment 2; III) mean results of experiments 1 and 2; IV) equation of antigenicity. Abscissa: doses of toxoid (first figures represent log of dose), ordinate: log LD_{50}.

$H = 3.348 + 0.548 \log D$, where H is the level of immunity and D the immunizing dose. It follows from this equation that when $D = 1$, $H = \log LD_{50} = 3.348$, and $LD_{50} = 2239$, i.e., after immunization with one antigen unit of toxoid the resistance of the mice is increased by 2239 times compared with unimmunized animals. Calculations showed that the limits of possible variations in the immunity level after immunization with one antigen unit may be from 794 to 6025 LD_{50}. Since the same toxoid was used for immunization of the animals and the same toxin for the reacting dose, these variations evidently depend entirely on differences in reactivity of the animals included in the experiment. These results confirm yet again that immunogenic properties of toxoids must be determined by comparison with a standard toxoid.

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