ENHANCEMENT OF THE ANTIGENIC ACTIVITY OF TOXOIDS BY SYMPATHOMIMETIC DRUGS

BY

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The immunization of children with fluid toxoids involves repeated injections of the antigen to achieve a satisfactory degree of protection. Research has been proceeding for some time into ways of improving antigenic efficiency, and a number of successful means have been found. Added water-insoluble mineral carriers—such as alum, aluminium or calcium salts, tapioca, oils and fats, and, lately, bacterial suspensions—have all had an enhancing effect on the antigenic efficiency of fluid toxoids. Their presence in prophylactic toxoids causes local irritation at the site of inoculation, the degree of irritation varying with the type of adjuvant used. It is very marked, for instance, with alum or insoluble calcium salts, but rather mild with bacterial suspensions.

For some years we have been interested in the problem of enhancing the antigenic action of toxoids, and to this end have so far been using *H. pertussis* suspensions with success. We realize, however, that there are occasions when the addition of pertussis vaccines is superfluous, as, for example, to toxoids required for booster doses to older children previously immunized against diphtheria, or to children known to be allergic, into whom the less foreign protein is injected the better.

Our attention has therefore been directed to drugs known to have a local tissue effect, either decreasing the rate of absorption or involving local inflammation. Of substances with the former pharmacological action we have studied the sympathomimetic drugs; with the latter, histamine. We have found that histamine had only a slightly stimulating action on the antigenic response to diphtheria toxoid, whereas the vasoconstrictor effect of the sympathomimetic group had a considerable enhancing effect.

As sympathomimetic drugs we used (1) adrenaline hydrochloride, at a dilution of 1/1,000 or 1/2,000; (2) benzedrine hydrochloride, at a dilution of 1/250; and (3) ephedrine hydrochloride, at a dilution of 1/250. In addition, two amines with no vasoconstrictor action, β -phenylethylamine (at a dilution of 1/250) and phenylethylamine (at the same dilution), were tested and found to be without stimulating effect on the antigenicity of diphtheria toxoid. The method of testing the different antigens was the same as used in earlier experiments (Ungar, 1954). Our new results are given in the Table.

Mean Antitoxin Titres in Groups of Ten Guinea-pigs After Giving Purified Diphtheria Toxoid with Various Substances

Group No.	Substance Added to Diphtheria Toxoid	Antitoxin Response (u./ml.) (Geometric mean of 10 animals)
1	Control. Plain toxoid	0.25
2	β -phenylethylamine 1/250	0.17
3	Phenylethylamine acetate 1/250	0.23
4	Benzedrine hydrochloride 1/250	1.96
5	Ephedrine hydrochloride 1/250	2.21
6	Adrenaline 1/1,000	2.87
7	Adrenaline 1/2,000	3.36
8	Histamine 0.5 μ g./ml.	0.70

Doses: 1 ml. diphtheria toxoid (2.5 Lf./ml.) injected by subcutaneous route. Two doses with four-weeks interval. Animals bled two weeks after second dose.

It should be noted that the amount of the vasoconstrictor added to the toxoid must be carefully chosen, for we have observed that higher concentrations of vasoconstrictors than those quoted above may cause local damage to the injected tissue.

From our results it may be concluded that the addition of vasoconstrictors had a considerable enhancing effect on the antigenic action of diphtheria toxoid. As adrenaline hydrochloride is stable only in an acid medium, it cannot be kept in contact with the toxoid for any length of time. On the other hand, ephedrine and benzedrine, being stable at a neutral pH, can be mixed with the toxoid and stored at a suitable temperature.

It seems that the addition of a vasoconstrictor to a toxoid might be useful for immunizing children against diphtheria; it has the additional advantage that its anti-allergic action would be of value for children subject to allergic manifestations after injections of antigens.

REFERENCE

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ACTIVITY OF PIPERAZINE, IN VITRO, AGAINST ASCARIS LUMBRICOIDES

BY

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Since the introduction and acceptance of piperazine as the drug of choice for the treatment of threadworms much interest has been taken in the possible value of this drug against other intestinal helminths. Piperazine hydrate and certain salts of piperazine have been shown to possess a high efficiency against *Ascaris lumbricoides* in man (Fayard, 1949; White, 1954; Brown, 1954; Brown and Serman, 1954; O'Brien, 1954; Rearden, 1954; Goodwin and Standen, 1954). Little work has been reported on the *in vitro* activity of piperazine against ascarides. Poynter (1955), using piperazine adipate *in vitro* against *Parascaris equorum*, showed that drug-affected worms recovered when they were placed in drug-free medium. The present series of experiments, described below, was carried out preparatory to trials with piperazine citrate against *A. lumbricoides* in man (Goodwin and Standen, 1954), and the experience so gained led to the observations made by these workers on the recovery of drug-affected worms passed by treated patients.

Materials and Method

The worms used in these experiments were *A. lumbricoides* from the pig. The culture medium was a modified Baldwin's solution, as described by Cavier and Savel (1952), in which these workers found it possible to keep ascarides alive for as long as 22 days. The composition of the medium was: NaCl, 0.8%; KCl, 0.02%; CaCl₂, 0.02%; NaHCO₃, 0.15%; MgCl₂, 0.01%; glucose, 0.5%; phosphate buffer to bring the solution to pH 8.4-8.6.

The worms were obtained from the abattoir, where they were removed from the gut immediately after the pig was slaughtered. They were placed at once in culture medium contained in a vacuum flask, and upon arrival at the laboratory were transferred to 8 by 5-in. (20 by 12.5-cm.) circular glass troughs containing fresh medium at 37° C. The worms were maintained overnight in this way, after which the most vigorous specimens of each sex were selected for experiment. For each experiment, two male and two female worms were placed in each trough and fresh medium was added to provide 125 ml. of fluid per worm. The drugs were added, in solution, to provide equivalent quantities of piperazine base as contained by 1:200, 1:280, 1:400, 1:560, 1:800, 1:1,120, and 1:1,600 dilutions of piperazine