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Disappearance of Human Diphtheria Antitoxin from Human Passive Transfer Skin Sites.* (26845)

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Kuhns(1) described a method for measuring the disappearance of human diphtheria antitoxin from passive transfer skin sites. The tests are carried out in Schick positive persons, and they depend upon intradermal antitoxin totally or partially to neutralize Schick toxin which is introduced later. Subsequent partial or total inhibition of toxic reactions indicate the degree of disappearance of antitoxin from skin sites. Further experiences with this method are described here. Precipitating, non-precipitating and skin sensitizing human antitoxic sera are compared as to their disappearance from passive transfer sites.

Materials and methods. Materials used in the Schick tests, evaluation of immediate reactions to Schick toxoid, and methods of hyperimmunization of Schick negative subjects have been described(2). Preparation and properties of the diphtheria toxin and toxoid used and the technics employed in carrying out the intracutaneous neutralization test in rabbits are described elsewhere(2,3,4). Human immune sera contained high titers of antitoxin as measured by rabbit skin tests. Precipitating and non-precipitating sera, and skin sensitizing and non-sensitizing sera have been used for purposes of comparison. Specimens from 6 individuals were utilized, designated as follows: Sp (non-precipitating – 15 Units/cc), She (precipitating – 80 Units/cc), Rie (precipitating – 50 Units/cc), Ni (non-precipitating – 100 Units/cc), Bo (precipitating skin sensitizing – 12 Units/cc), and Hu (non-precipitating skin sensitizing – 20 Units /cc). All serum samples to be tested were removed from the frozen state at 0°F and rapidly thawed to room temperature just prior to use. Dilutions of antitoxin were made in borate-NaCl buffer pH 7.4 containing 200 μg gelatin/cc to prevent surface denaturation. Methods of quantitatively measuring precipitation in a fluid and semi-solid medium have been described(5). Wheal reactivity was measured by the method of passive transfer (6). Toxoid and toxin preparations from the same source were employed for all testing purposes.

Neutralization of Schick Toxin by Intradermal Antitoxin: This test is based upon the ability of intradermal antitoxin to neutralize the toxic action of the equivalent amount of Schick toxin in Schick positive subjects. Recipients were used for no more

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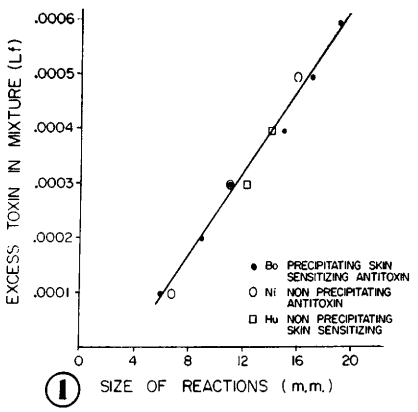
than 2 series of tests spaced about one week apart. Circulating antitoxin titers were carried out on blood specimens obtained at time of testing. Recipients who demonstrated a changing circulating antitoxin titer were not employed for additional tests. When successive series of passive transfer tests were carried out in one person, sites of earlier testing were avoided. Excellent agreement in results was obtained in testing in duplicate skins when both recipients were Schick positive.

Skin disappearance of antitoxin was measured as follows: Control experiments were run in which separate mixtures containing both Schick toxin (0.0006 Lf) and different amounts of antitoxin (0.0001 to 0.0012 Unit) were placed at separate intradermal sites following incubation at 37°C for one hour. If toxic reactions occurred, measurements were made (diameter in mm) 4 days later. Each measurement provided an indication of the free toxin which remained in such mixtures, permitting direct correlation of this information with size and intensity of the toxic reaction. The data in turn were applied to other experiments where first antitoxin and then toxin were injected at variously spaced intervals at the same sites. A toxic reaction measured as described above served as an indication of toxin in excess of the amount which was bound to intradermal antitoxin. The amount of antitoxin bound to toxin in the skin was then estimated assuming 1 Lf = 1 Unit of antitoxin. On the basis of difference it was also possible to estimate the amount of antitoxin which disappeared between sensitization and antigen challenge at the same site. In the present experiments, the amount of antitoxin used to sensitize a skin ranged from 0.0003 Unit to 1.2 Unit contained in 0.1 cc. Challenge with 0.1 cc (0.0006 Lf) of Schick toxin was at intervals ranging from 6 hours to 7 days.

For each antitoxic serum or serum dilution which was injected alone prior to toxin challenge, a control containing the same amount of antitoxin in a mixture with toxin was injected simultaneously in the same recipient.

Results. A straight line relationship was observed between size of reactions and amount of excess toxin in mixtures containing Schick toxin (0.0006 Lf) and different amounts of antitoxin ranging from 0 to 0.0005 Unit (Fig. 1). Precipitating, nonprecipitating and skin sensitizing antitoxins all exhibited the same relationship when employed at comparable strengths in mixture with toxin. When antitoxin was injected separately followed by toxin later at the same skin sites, reactions undiminished in size and intensity from toxin controls at unsensitized sites were indicative of the disappearance of all antitoxin between sensitization and challenge. Attenuated reactions, depending upon



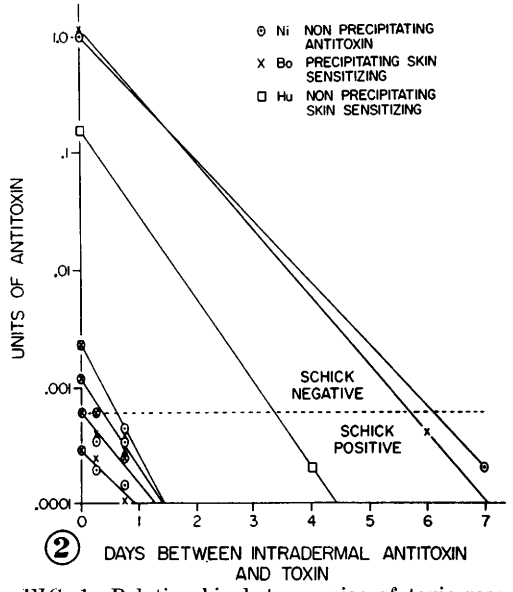


FIG. 1. Relationship between size of toxic reactions and excess toxin in mixtures containing Schick toxin and antitoxins in different amounts.

FIG. 2. The disappearance of human diphtheria antitoxin from human passive transfer sites.

TABLE I. Disappearance of Human Antitoxin Bo in Skin of Schick Positive Recipient Judged According to Ability to Neutralize Schick Toxin.

Units of antitoxin at skin site	Time of challenge with .0006 Lf Schick toxin, hr	Toxic reaction 4 days after challenge, mm intensity		Calculated free toxin (Lf) causing reaction	Calculated antitoxin (units) Remaining at time of challenge	Calculated antitoxin (units) Disappeared between skin test and challenge
.0024	6	0		0	>.0006	<.0018
.0012	"	5	±	Little or 0	ca. .0006	ca. .0006
.0006	"	9	++	.0002	.0004	.0002
.0003	"	13	+++	Between .0003 & .0004	Between .0002 & .0003*	.0001
.0024	18	9	+++	.0002	.0004	.0020
.0012	"	11	+++	.0003	.0003	.0009
.0006	"	13	++++	Between .0003 & .0004	Between .0002 & .0003	Between .0003 & .0004
.0003	"	17	++++	.0005	.0001	.0002

* A point midway between these figures is selected for purposes of charting.

size and intensity, represented a partial disappearance of antitoxin (Table I).

Rate of disappearance of intradermal antitoxin was determined. In one set of experiments, the amount of intradermal antitoxin very closely approximated the dose of Schick toxin employed later. Accordingly the following amounts of antitoxin were placed at intradermal sites 4 to 5 cm apart on the back in 2 different Schick positive recipients; 0.0003, 0.0006 and 0.0012 Unit. Skin sites were prepared in this fashion in duplicate so that toxin challenge of a single series could be carried out at 6 hours and at 18 hours after sensitization. Toxic reactions at test sites were compared with reactions at control areas (see under Methods) and antitoxin which remained at test sites was estimated as described previously. Results of such an experiment are indicated in Table I. Sites challenged at 6 and 18 hours were found to contain residual antitoxin, but smaller amounts of antitoxin were present upon toxin challenge at 18 hours as compared with challenge at 6 hours. The antitoxin initially utilized, together with antitoxin residuals at 6 and 18 hours were then plotted as a function of time to obtain the rate of disappearance of this antibody. On the basis of the above experiments, it was estimated that 50% of diphtheria antitoxin disappeared from intradermal sites in 10 to 14 hours when 0.0003 and 0.0006 and 0.0012 Units of intradermal antitoxin were used (Fig. 2).

In other experiments, sensitization with intradermal antitoxin was carried out using amounts far in excess of the amount equivalent to the dose of Schick toxin employed later. 0.0024 Unit, 0.15 Unit, 1.0 Unit and 1.2 Units of antitoxin contained in 0.1 cc were placed in replicate spaced intradermal sites in the back of Schick positive subjects. Later challenge with Schick toxin (0.0006 Lf) was made when the residual intradermal antitoxin was believed to be 0.0006 Unit or less (in the case of 0.0024 Unit, times of challenge were 6 and 18 hours). A suitable time interval could usually be extrapolated from experiments in which equivalent antitoxin and toxin were utilized based upon an appropriate multiple of the time required for disappearance of antitoxin under these circumstances (Fig. 2).

Under the conditions of these experiments 50% of the antitoxin disappeared from skin in 10 to 14 hours when 0.15 Unit, 1.0 Unit and 1.2 Units were initially used. A 50% disappearance rate of 7 hours was obtained when 0.0024 Unit of antitoxin was employed.

Non-precipitating antitoxin, precipitating and skin sensitizing antitoxins all behaved similarly in these experiments (Fig. 2, Table I).

Discussion. The disappearance of human diphtheria antitoxin was studied in human passive transfer skin sites using a method which depended upon intradermal antitoxin to neutralize Schick toxin totally or in part.

Schick positive recipients were required for these experiments. Intradermal antitoxin in sufficient amount in these persons produced local areas which were Schick negative. The extent of disappearance of intradermal antitoxin was calculated following reversion to the Schick positive state when comparison of the toxic reaction could be made with reactions produced by standard toxin-antitoxin mixtures. All calculations of antitoxin were made according to the formula 1 Lf toxin = 1 Unit antitoxin. Loss of antitoxin was then estimated by subtracting residual antitoxin (as calculated above) from the antitoxin initially used. Estimates of rate of disappearance were made by plotting initial *vs.* residual antitoxin as a function of time.

It was found that 50% of antitoxin disappeared from skin sites in 10 to 14 hours when the following amounts of intradermal antitoxin were employed; 0.0003 Unit, 0.0006 Unit, 0.0012 Unit, 0.15 Unit, 1.0 Unit and 1.2 Units. A 50% disappearance rate of 7 hours was obtained when 0.0024 Unit was used. On the whole the results employing various amounts of antitoxin were in good agreement, and non-precipitating, precipitating and skin sensitizing antitoxin behaved similarly under conditions of the test.

The tendency to variability in this figure for antitoxin excess may be explained as follows. Under this circumstance the skin was not reactive to Schick toxin during most of the time that intradermal antitoxin was migrating away. Any data on antitoxin disappearance were of necessity derived from gradations in toxic reactions over a short period of time during which time only a small part of the original antitoxin remained. The fraction of antitoxin which remained was not known exactly but was extrapolated from disappearance rates of much smaller *i.e.*, equivalent amount of antitoxin. This ordinarily allowed for a single challenge following sensitization. Curves constructed from these data were thus based on 2 points (Fig. 2). On the other hand, additional points, particularly in the immediate period following sensitization, were possible when intradermal antitoxin in the region of equivalence was employed. Disappearance after 18 hours could not be tested because of difficulties of interpreting small differences between control and sensitization sites.

Other technics and immune systems have been used to study antibody metabolism in skin. Farah, Kern and Eisen(7) studied the disappearance from human skin of purified rabbit anti-DNP antibody and the fragments split from this antibody by papain. Intradermal injections were made using I^{131} labelled antibody or antibody fractions, and counts of radioactivity were used to measure loss of antibody from the skin. The results indicated a half life of approximately 10 hours for intact antibody and antibody fractions. Humphrey, Neuberger and Perkins (8) also utilized radioactive isotopes in experimental animals to calculate the exchange between skin albumin and circulating albumin. Their data suggested that 60% of skin albumin is replaced every day by circulating serum albumin.

The findings for disappearance of antibody in human skin are consistent with our results. The results suggest that, for the systems employed, disappearance of antibody from human skin occurs at a relatively constant rate, and is not apparently related to differences in molecular weight, precipitability, or immediate wheal activity.

The method described here may be useful in further investigations on the nature of reagins. Purified systems must be employed to study further comparisons between the wheal reactive state and other criteria of antibody activity in the skin as described herein, to assess the significance of earlier findings (1) that reagin persists for an unusually long period *in vivo*.

Summary. This paper has described further experiences with a passive transfer technic which depends upon the ability of intradermal antitoxin to neutralize Schick toxin introduced simultaneously or later at the same sites. Partial, total or no inhibition of toxic reactions indicates the amount of antitoxin remaining at the skin sites. The amount of antitoxin which has disappeared may be calculated by difference, assuming that 1 Lf toxin = 1 Unit of antitoxin. On the basis of this test 50% of antitoxin intro-

duced at skin sites was generally found to disappear in 10 to 14 hours. No difference in disappearance from the skin was observed between precipitating antitoxin, non-precipitating antitoxin and skin sensitizing antitoxin.

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Effect of Topical Application of Hydrocortisone on Dermic Lesions Produced in Rabbits by Vaccinia Virus. (26846)

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Cortisone and its derivatives are commonly employed as topical medication in several skin and mucosal diseases. Inflammatory reactions are thereby diminished and symptoms such as rash and pruritus are alleviated in many cases. However, in other cases local application of cortisone involves hazards which it is important to consider. These hazards occur in some viral diseases, among which those produced by herpes simplex virus are good examples. In fact, in man, conjunctival lesions of such an origin grow and deepen as a result of the local action of corticoids, as has been observed in experimental infection of the rabbit(1,2). Trachoma is also aggravated during the acute phase or may be reactivated during later stages when treated locally with those substances(3). Nevertheless, the literature does not mention similar observations in relation to other viruses that produce epidermal lesions.

In this work the local effect of hydrocortisone on dermal lesions produced in the rabbit by vaccinia virus is studied.

Material and methods. The strain employed belonged to the Instituto Nacional de Higiene virus seed whose lots 32 and 38 of vaccinia virus were prepared in calves. In the lymph potency test, determined by Force and Leake's technic(4), confluent lesions were observed at 1:10,000 dilution of lymph and isolated lesions at 1:30,000.

Once the inoculum potency had been determined, 24 healthy albino rabbits were selected at an average weight of 4,500 g. Two areas on the back of each rabbit, measuring 4 cm $\times$ 4 cm and situated 5 cm apart, were depilated. One percent hydrocortisone ointment was applied on one of the areas and neutral vaseline on the other. Both applications were performed daily. Six days after the treatment had begun the rabbits were inoculated intracutaneously through scarification with 0.3 ml of a viral suspension diluted 1:30,000 in saline. The inoculum was carefully spread throughout the area. Two hours later, when the fluid had thoroughly soaked the skin, ointments were applied and the treatment was continued for 6 days more.

To detect the appearance of vesicles the rabbits were observed daily until maximal development of lesions. As soon as the vesicles appeared they were measured every 24 hours by applying a glass slide over the affected