PROGRESS IN PEDIATRICS

ACTIVE IMMUNIZATION AGAINST DIPHTHERIA BY MEANS OF VON BEHRING'S VACCINE, AND THE DIPHTHERIA TOXIN SKIN REACTION *

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I. ACTIVE IMMUNIZATION

The weak point in the immunizing use of diphtheria antitoxin lies in the short duration of the passive immunity thus conferred. The injected antitoxin is rapidly broken down and eliminated until in the average case there is not sufficient antitoxin left at the end of ten days to afford further protection. (Von Behring, Park.) While this ten day period may be enough to protect the average individual who has been in contact with a case of diphtheria, it is not adapted to protect those who are constantly or repeatedly exposed to infection, as for example nurses or physicians on service in a diphtheria ward, or patients in the wards of a children's hospital. The rapid destruction and elimination of antitoxin is due in large part to the use of a serum obtained from an animal of a foreign species. Park found that if a guinea-pig received 10 units of diphtheria antitoxin obtained from a horse (heterologous serum) there was only $\frac{1}{2}$ unit left at the end of seven days and $\frac{1}{20}$ of a unit at the end of fourteen. When, on the other hand, a guinea-pig was given 10 units of guinea-pig antitoxin (homologous serum) there was 1 unit left at the end of fourteen days. Matthes gave a 4 kg. child 350 units of an homologous serum. Following the injection the content of the blood serum in antitoxin reached a height of $\frac{1}{5}$ units per c.c., and one month later $\frac{1}{20}$ of a unit per c.c. could be demonstrated.

In practically every non-fatal case of diphtheria there is an active production of antitoxin as a result of the reaction of the body to the absorbed diphtheria toxin, which persists for a varying length of time. Park mentions a case of diphtheria in which the blood showed 60 units of antitoxin per c.c. at the end of the fifth day, 90 per cent. of which was produced by the patient. The average case forms very much less than this. In this connection the findings of Kissling are most interesting. Kissling studied the production of antitoxin in physicians and nurses working in diphtheria wards. Those who had never had clinical diphtheria but who had been working about the wards for a long time

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showed high amounts of antitoxin in their blood serum, while those recently assigned to duty showed low amounts and frequently contracted the disease. In other words an insensible active immunization had gradually taken place in those who had been on duty in the diphtheria wards for some length of time. The recent work of Schick and his coworkers would seem to show that active immunization is an exceedingly common occurrence, and that a large percentage of individuals—although they may never have had clinical diphtheria—possess sufficient antitoxin to protect themselves against infection except probably during severe epidemics.

The idea of the production of active immunization for purposes of prophylaxis is by no means new, and has been suggested by a number of workers in this field in past years. In 1909 Theobald Smith reported that he had obtained an active immunization in guinea-pigs, which lasted over several years, by the injection of toxin-antitoxin mixtures. Mixtures containing a slight excess of toxin produced the best immunity. Smith at this time suggested the use of toxin-antitoxin mixtures in man, and recognized the limitations of such a method. He found that diphtheria toxin alone produced a very slight degree of immunity with severe local lesions. Later, together with Brown, he reported the production of immunity in guinea-pigs by the injection of toxin in amounts so small that local necrosis did not occur. The immunity obtained in this way was sufficient to enable the guinea-pig to withstand one-half the lethal dose without a local reaction.

The first attempt to produce active immunization in man by means of toxin-antitoxin mixtures was reported at the 1913 meeting of the Kongress für Innere Medizin at Wiesbaden by Hahn, who presented the results of the use of such a vaccine prepared by von Behring at Marburg, and during the past year a number of papers on this subject have been published by von Behring and his associates. While the idea of active immunization by toxin-antitoxin mixtures is not new, as we have shown, von Behring claims originality through the results of his studies with such mixtures. It is not within the scope of this review to enter into a discussion of the relationship and combination of toxin and antitoxin, and we simply state von Behring's claim of the reversibility of such combinations. He found that a mixture in which 1 unit of toxin was neutralized by 1 unit of antitoxin for the guinea-pig, was not necessarily atoxic for other species of animals. Thus if 1 unit of toxin is combined with 20 to 40 units of antitoxin and the mixture injected into a monkey, the toxin causes death, and it is necessary to use 80 to 100 guinea-pig antitoxin units to neutralize 1 unit of toxin for the monkey.

The so-called "von Behring's vaccine" consists of a mixture of strong diphtheria toxin and antitoxin in such proportions that the toxin

is just neutralized, or is in very slight excess, when tested on a guinea-pig. A number of different mixtures have been used which are designated as M 1/M 11, MM 1, etc., and which vary somewhat in their proportions. The mixtures remain active for ten weeks after preparation. At first the vaccine was injected subcutaneously and the formation of antitoxin in the blood serum studied by Römer's method. It was found that from twenty to twenty-five days elapsed before the development of immunity.¹ It was later found that if the injections were given intracutaneously a stronger immunity resulted and the interval between the injection and the development of immunity was reduced to ten days. This largely overcomes the most serious practical objections to the use of the vaccine.

The vaccine produces a reaction at the site of injection which is similar to the local tuberculin reaction. By testing the blood of vaccinated individuals it has been found that the degree of the local reaction corresponds to the amount of antitoxin formed, and hence furnishes a simple and reliable index of the degree of immunization produced. It is impossible to test the blood for its antitoxin content where a number of people are vaccinated, and Hahn and Summer have put this phenomenon to practical use by recording the degree of immunization according to the degree of the local reaction, and have elaborated a scheme for recording the local reaction according to the area and degree of reddening, infiltration, and scaling that occurs. According to Kleinschmidt and Viereck the local action of the toxin of the vaccine does not depend on the antitoxin in the blood, as is the case when diphtheria toxin alone is injected intracutaneously. A negative reaction with the latter is evidence of antitoxin in the blood, and it has frequently been observed that individuals with antitoxin in the blood are more susceptible to the vaccine than those without. Antitoxin in the blood—which depends on an active immunization from previous infection and which is found in some 50 per cent. of people—apparently leads to a hypersensitiveness toward the toxic agent. Kratz has shown that a toxin-antitoxin mixture which is toxic for an actively immunized animal is neutral for a control animal of the same species. It is suggested that a previous active immunization leads to a more rapid cleavage of the toxin-antitoxin mixture.

Hahn in his original report gave the results of the use of the vaccine in forty cases. No bad results were noted. The formation of antitoxin was studied in all of these cases which are largely

1. The antitoxin content of the blood serum which affords immunity apparently lies between 1/10 and 1/30 units per c.c., except perhaps in case of infection with organisms of high virulence.

of theoretical interest. Matthes likewise studied the formation of antitoxin. The results in one case (K) were as follows:

December 23, K. was given 0.25 c.c. of MM 1/4 subcutaneously. Previous to vaccine injection the blood showed an antitoxin content of $\frac{1}{8}$ units per c.c. Later tests were as follows:

10 days after injection—160 units of antitoxin per c.c.
16 days after injection—175 units of antitoxin per c.c.
21 days after injection—20 units of antitoxin per c.c.
12 weeks after injection—8 units of antitoxin per c.c.

(v. Behring reckons that it will be two years before antitoxin falls to 1/100 units per c.c., in this case.)

Zangmeister reported that new-born children do not react to large doses of the vaccine. Kleinschmidt and Viereck studied the antitoxin formation in twenty-seven children and came to the conclusion noted above, that intracutaneous injections are more active and that the degree and rapidity of immunization corresponds to the strength of the local reaction.

Following these rather theoretical studies, in which no bad results were observed, Kissling, and Hahn and Summer, used the vaccine in a large number of cases in order to test its practical value. Kissling injected 310 patients in the wards of his hospital at Hamburg who had been exposed to diphtheria. Of these 111 received two injections and no cases of diphtheria developed after vaccination. One hundred and ninety-nine were injected once and eight of these subsequently developed clinical diphtheria. The cases which developed were in the scarlet fever and pertussis wards.

Scarlet fever: 197 patients were immunized; 108 injected twice; 89 injected once; 5 cases developed.

Of these 1 developed on the fourth day after injection; 1 on fifth day after injection; 1 on nineteenth day after injection; 1 on thirty-fifth day after injection; 1 on thirty-seventh day after injection. The last three were very mild.

Pertussis: Thirty cases immunized; three cases of diphtheria developed.

Kissling observed four severe reactions when the vaccine was injected into the arm and prefers to inject into the skin of the back along the line of the scapula.

Hahn and Summer tried out the vaccine in certain villages and small cities where diphtheria was epidemic. They found a tremendous difference in the susceptibility of different individuals to the vaccine, some reacting less than others to doses fifty times greater. For this reason they first inject a small dose (0.01 c.c. intracutaneously) and if a severe reaction is obtained a second injection is not given, as the first was found to give satisfactory immunity. If a mild reaction is obtained, a second injection with a doubled dose is given on the third day, and if no reaction followed the first injection a 10-times dose is given at the second injection. They advise two or even three

injections a day apart unless the first or second reactions are very severe. Children were vaccinated in five villages and cities and as many did not return for examination after the first or second injections, the cases are divided as follows:

1. Complete immunization: good repeated reaction.
2. Doubtful immunization: slight reaction after first and did not return after second injection.
3. Unsatisfactory immunization: did not return for examination after first injection.

An example of their results is as follows:

CITY OF EGELN (5,300 INHABITANTS)

Cases from January 12 to November 11.....	=72	} 7.5 per cent. mortality.
First immunization, November 11.....		
Cases November 11 to December 18.....	=27	
	Total No. Children	Cases of Diphtheria which Developed after November 11
Non-immunized	1,089	21
Inside first ten days.....		4
Unsatisfactory immunization	89	0
Doubtful	130	1
Complete	304	1

Thus 20 per cent. of the non-immunized developed diphtheria and 10 per cent. of the immunized. If the four cases developing in the first ten days are omitted, the percentage of immunized cases is only 3.3. Of these four cases, one was so mild that antitoxin was not used. The totals were as follows:

	Total No. Children	Cases of Diphtheria
Complete immunization	633	2
Doubtful immunization	255	2
Unsatisfactory immunization	209	1
Cases developing in first ten days after injection.....		10
Cases developing among non-immunized.....		51

Of the two cases developing among the "completely immunized," one was abortive and the other was a clinical case without bacteriological control.

A "negative phase" between the time of the injection and the development of immunity has not been noticed by any who have used the vaccine so far. On the contrary Hahn and Summer note that the ten cases of their series which developed within ten days after injection were all characterized by a mild abortive course. The antitoxin portion of the vaccine has not produced an hypersensitiveness to further injections of antitoxin used for curative purposes in those patients who developed diphtheria subsequent to the injection of the toxin-antitoxin mixture.

The vaccine is still in an experimental stage and its final value and usefulness remain to be determined. So far no bad or unpleasant results have been reported. If further use supports these early impressions the vaccine will probably find a definite place in the

prophylactic treatment of diphtheria, as numerous indications for the use of such a means occur at once to the clinician.

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II. THE DIPHTHERIA TOXIN SKIN REACTION

NATURE OF THE REACTION

In 1909 Schick found that if a solution of diphtheria toxin was applied to the skin in the same manner that tuberculin is used in the von Pirquet tuberculin test, it produced a reaction in many persons very similar to the tuberculin reaction. Later, by using the intracutaneous method, he was able to regulate the amount of toxin absorbed and this has led to a number of studies which are of both theoretical and practical importance.

The toxin used by Schick and his associates is a dilution of such strength that 0.1 c.c. equals 1/50 of the lethal dose for a 250 gm., guinea-pig. The lethal dose of the toxin which Schick uses is 0.005, and hence he injects 0.1 c.c. of a 1-1,000 dilution. In those who react an area of reddening and infiltration develops within twenty-four hours, reaching its maximum in forty-eight hours, and which heals with scaling and a characteristic central pigmentation. Although the reaction is similar to the local tuberculin reaction, its interpretation is directly opposite. The diphtheria toxin is a direct toxic agent and by control tests of the blood serum it has been found that *a negative reaction is always associated with the presence of diphtheria antitoxin in the blood* of the person tested. While

as a rule a positive skin reaction is an indication of the absence of antibodies, some persons react positively for some unexplained reason who possess a greater amount of antitoxin in the blood than 0.03 units per cubic centimeter.

It has been found that if a negative reaction follows the injection of a 0.1 c.c. of a 1-1,000 dilution of toxin, the individual tested has at least 0.031 units of antitoxin per c.c. in his blood when tested by Römer's method. A person with a higher concentration of antitoxin will react negatively to a smaller dilution of antitoxin and vice versa. Thus the outcome and the degree of reaction are dependent on two factors—the strength of the toxin used and the presence of antitoxin in the blood.

The specificity of the diphtheria toxin skin reaction is shown by its suppression or inhibition by the simultaneous injection of antitoxin in individuals who have previously given a positive reaction. In this connection the work of Schick and Maygar, who studied the simultaneous injection of diphtheria toxin and tuberculin, is interesting. Previous studies of tuberculin and toxin reactions in the same individual by Rolly, Entz and others, led them to conclude that the reaction to tuberculin was a general reaction toward toxic substances. Schick and Maygar tested 315 individuals with both tuberculin and diphtheria toxin at the same time and only obtained the same reaction (both positive or both negative) in 163 cases. In 153, or almost 50 per cent., the reactions were different.

THE REACTION AS A PRELIMINARY TEST FOR PROPHYLACTIC INJECTIONS OF ANTITOXIN

As noted above, it has been found that if a negative reaction follows the injection of 0.1 c.c. of a 1-1,000 dilution of toxin, the blood serum contains at least 0.031 units of antitoxin per cubic centimeter. This is sufficient to afford immunity against infection with the diphtheria bacillus under ordinary circumstances.

A large number of tests on normal individuals have been made and the reactions obtained are given in the following tabulation. Many of these have been controlled by Römer's method.

	Total	Positive	Negative	Per Cent. Negative
New-born infants.....	291	10	275	93
First year of life.....	42	18	24	57
2 to 5 years.....	150	95	55	37
5 to 15 years.....	264	131	133	50

It is thus seen that a large percentage of individuals are immune to diphtheria. The age period giving the lowest percentage of negative reaction (2 to 5 years) is the same as that in which the highest incidence of clinical diphtheria is found. As far as could be deter-

mined the reaction remains the same for a period of at least four weeks.

The practical value and application of such results is obvious. Whenever it is necessary to immunize against diphtheria for prophylactic purposes, a diphtheria toxin skin test will show in twenty-four hours those who already possess immunity. Only those reacting positively to the toxin require passive immunization with antitoxin. As roughly 50 per cent. possess immunity this will reduce the number of prophylactic injections one-half. The real extent of such a reduction is seen when one considers that, according to Park, two injections for immunization are given in New York City to one for curative purposes. The liability to sensitization to horse serum is coincidentally lessened to the same extent.

AS A TEST FOR ANTITOXIN CONTENT OF THE BLOOD

In the past the manner of measuring the quantity of diphtheria antitoxin in the blood has been indirect. The blood serum of the individual tested has been added in different dilutions to a definite quantity of toxin and injected into guinea-pigs. The blood content in antitoxin is calculated from the dilution which just neutralizes the toxin.

Schick and Michiels having once found that 0.1 c.c. of a 1-1,000 dilution of their toxin requires 0.031 units per cubic centimeter of toxin in the blood to suppress the toxin reaction, have been able to test directly the quantity of antitoxin by varying the strength of the dilution. If a negative reaction follows a 1-1,000 dilution, a 1-500, 1-200, etc., dilution was used until a strength was reached at which a positive reaction occurred. It is necessary first to find the strength of a toxin on guinea-pigs, after which it may be used directly in different dilutions.

USE IN ANTITOXIN EXPERIMENTS

Until the elaboration of the toxin skin reactions, all experimental work in regard to the relations of toxin to antitoxin have been carried out on animals, and our ideas regarding the administration and efficacy of antitoxin in man have very largely been based on animal experimentation. Schick and his associates have carried out a large number of experiments on children in regard to these questions.

Children who reacted positively to a toxin skin test were given antitoxin in different amounts and at the same time a toxin reaction was made. Fairly large quantities of antitoxin were needed to suppress the reaction (2,000 units intramuscularly). In another series of experiments toxin tests were made at two and three hour and

longer intervals and the effect of large doses of antitoxin noted. Practically no effect could be made on toxin injected more than three hours before the administration of antitoxin. Reactions from injections of toxin made forty-eight, seventy-two and ninety-six hours after the administration of fairly large quantities of antitoxin were suppressed.

Schick comes to the conclusion that the action of antitoxin is chiefly immunizing, and that injections should be made as early as possible and either intravenously or intramuscularly. The value of antitoxin in severe cases depends on whether a lethal dose of toxin has been absorbed before the antitoxin was administered.

OTHER USES

A few attempts have been made to produce active immunity by repeated injections of small amounts of toxin. Three injections of 0.1 c.c. of a 1-500 dilution were made two weeks apart. As no weakening of the reaction was observed at the third injection the result was looked on as unsuccessful. No indications of hypersensitiveness were observed.

As there is no antitoxin present free in the blood in acute diphtheria, the use of the reaction for diagnostic purposes has been suggested. Thus in a suspected case or questionable diagnosis a negative reaction—indicating the presence of antitoxin—would speak against the diagnosis of diphtheria.

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