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Research Article

THE INFLUENCE OF DOSAGE AND ROUTE OF INJECTION ON THE ANTIBODY RESPONSE OF HUMAN SUBJECTS TO THE SPECIFIC CARBOHYDRATE OF THE TYPE VIII PNEUMOCOCCUS [1]

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The readiness with which immune bodies could be demonstrated in human subjects following the injection of the specific carbohydrate of the Type VIII pneumococcus (1) offered an opportunity to study, in man, the effect of dosage and of the route of injection upon antibody production with a carbohydrate antigen. Although numerous investigators have made similar studies with whole bacterial antigens in lower animals and in man, no data are available concerning the comparative antigenic activity, in man, of purified bacterial fractions given in different amounts or by different routes. The development of antibodies following injections of type-specific polysaccarides was first demonstrated by Francis and Tillett (2) in the course of their investigations on cutaneous reactions in lobar pneumonia.

EXPERIMENTAL

Subjects, materials and methods

The subjects chosen for this investigation, the Type VIII pneumococcus specific carbohydrate used, and the methods employed for the antibody studies have been described in the previous communication (1). Except in the subjects included in that report, phagocytic tests were performed irregularly. Only the results of the tests for agglutinins and mouse protective antibodies, therefore, will be considered here.

The intracutaneous injections were made into the volar aspect of the forearm, the subcutaneous ones over the deltoid insertion, and the intravenous doses were given into the antecubital vein. Because antibodies were often stimulated by minute amounts of the carbohydrate given intracutaneously, special precautions were taken, in making the intravenous injections, to avoid getting any of the material into or under the skin. This was done by attaching the syringe containing the carbohydrate solution to the needle only after a free flow of blood was obtained, and then, following the injection, drawing some blood into the syringe before the needle was withdrawn. All of the injections were made with the carbohydrate in solution in physiological saline which was especially prepared (3). The intracutaneous injections were made in 0.1 cc. amounts. All other injections were in 1.0 cc. amounts except the 5.0 mgm. doses, which were given in a volume of 1.25 cc. No local or general reactions followed any of the injections.

RESULTS

In Table I, in the previous communication (1), there are tabulated the homologous type-specific antibody titers of each of the bloods obtained from subjects who received 1.0 mgm. of the Type VIII carbohydrate subcutaneously. The results of the studies of the individual sera in the remainder of the subjects now under consideration corresponded, in a general way, to those shown in that table. For the sake of brevity, and because the major interest in this paper lies in a comparison of the antigenic effects that variations in the dose and route of injection have upon the development of antibodies, only the antibody titers of the sera obtained before injection and the ones showing the maximum titers after injection in each subject will be noted. These titers in each of the individuals receiving various doses of the carbohydrates of the Type VIII pneumococcus by the intravenous, subcutaneous or intracutaneous route are shown in Table I. The maximum accretions in titer of agglutinins and mouse protective antibodies are represented graphically in Figures 1 and 2, respectively, in such a way as to make possible a comparison of the antibody response to each of the various doses. While the number of subjects in each group is small, the trends are apparent and worth noting.

The results of the studies, shown in the table and figures, may be summarized briefly: 1. Each of the doses tested, which ranged from 5.0 mgm. to 0.001 mgm. intravenously, from 1.0 mgm. to 0.001 mgm. subcutaneously, and from 0.15 mgm. to 0.0001 mgm. intracutaneously, stimulated in some subjects an appreciable titer of the homologous type-specific antibody. 2. In general, regardless of the route of injection, when decreasing

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TABLE I

Homologous type antibody response to single injections of Type VIII soluble specific substance

Subject	Sex	Age	Amount injected	Agglutinins (serum dilution)		Mouse protection (fatal doses per 0.2 cc.)	
				Before	After	Before	After
		years	*mgm.*				
A. Intravenous							
H.J.B.	M	56	5.0	1 : 2	1 : 64	100	1,000,000
J.B.	M	45	5.0	0	1 : 8	1,000	1,000,000
M.A.	F	79	5.0	0	0	0	0
T.N.	M	69	5.0	0	0	0	0
I.C.	F	44	5.0	0	0	0	0
F.K.	M	61	5.0	0	0	0	0
C.D.	M	54	1.0	0	1 : 16	0	100,000
W.P.B.	M	59	1.0	0	1 : 8	0	100,000
T.T.	M	73	1.0	0	1 : 128	100	100,000
C.McF.	F	29	1.0	0	1 : 4	0	100,000
D.B.	F	65	1.0	0	1 : 16	10,000	1,000,000
D.L.	M	71	1.0	0	1 : 4	0	1,000
T.R.	M	24	1.0	0	1 : 4	0	1,000
A.C.	F	73	1.0	0	0	0	10
F.B.	M	50	0.1	0	1 : 64	0	100,000
C.B.	M	59	0.1	0	1 : 2	0	100,000
G.M.	M	56	0.1	0	1 : 2	0	100,000
J.M.	M	51	0.1	0	1 : 16	1,000	1,000,000
H.S.	M	49	0.1	0	1 : 4	0	10
M.L.	M	58	0.1	0	0	0	0
J.P.	M	46	0.01	0	1 : 8	0	1,000
C.C.	M	72	0.01	0	0	0	1,000
J.G.	M	76	0.01	0	1 : 4	100	1,000
T.F.	M	13	0.01	0	0	0	0
E.B.	M	24	0.01	1 : 4	1 : 4	100	100
J.K.	M	59	0.01	0	0	1,000	100
C.Ca.	M	21	0.001	0	1 : 4	0	1,000
E.L.	M	25	0.001	0	1 : 2	10	10,000
V.B.	M	45	0.001	0	0	0	0
C.M.	M	37	0.001	1 : 4	1 : 2	10,000	10,000
F.T.	M	39	0.001	0	0	0	0
B. Subcutaneous							
M.K.	F	62	1.0	0	1 : 32	0	1,000,000
A.S.	M	22	1.0	0	1 : 8	10	10,000,000
F.S.	F	39	1.0	0	1 : 16	0	1,000,000
M.McG.	F	35	1.0	0	1 : 16	100	10,000,000
F.C.	M	45	1.0	0	1 : 4	0	1,000,000
W.H.M.	M	68	1.0	0	1 : 4	10	1,000,000
S.V.C.	F	79	1.0	0	1 : 4	10	1,000,000
J.N.	M	40	1.0	0	1 : 8	0	100,000
J.P.C.	M	62	1.0	0	1 : 16	10	100,000
E.J.B.	M	75	1.0	1 : 2	1 : 4	10	100,000
K.R.	M	61	1.0	0	1 : 4	0	1,000
S.B.	F	59	1.0	0	1 : 8	0	100
J.H.	M	25	0.1	0	1 : 32	0	100,000
J.McC.	M	52	0.1	0	0	0	100,000
W.C.	M	51	0.1	0	1 : 4	0	10,000
F.M.	M	14	0.1	0	0	0	1,000
C.B.	F	39	0.1	0	0	0	100
F.W.	F	24	0.1	0	0	10,000	100,000
E.R.	M	32	0.01	0	1 : 4	0	100,000
J.D.S.	M	68	0.01	0	1 : 2	0	100,000
A.G.	M	32	0.01	0	1 : 4	0	1,000
J.G.	M	49	0.01	1 : 4	1 : 32	100,000	1,000,000
W.H.	F	60	0.01	0	0	10	1,000
M.P.	F	50	0.01	1 : 2	1 : 2	10	10
P.K.	M	75	0.01	0	0	0	0
M.T.	F	50	0.001	0	1 : 8	0	100,000
M.R.	F	36	0.001	0	0	0	1,000
F.J.	F	58	0.001	0	0	10	1,000
M.F.	F	21	0.001	0	0	0	100
P.G.	F	26	0.001	0	0	10	100
E.M.	M	60	0.001	0	0	0	0
M.C.	F	66	0.001	0	0	0	0

TABLE I (*continued*)

Subject	Sex	Age	Amount injected	Agglutinins (serum dilution)		Mouse protection (fatal doses per 0.2 cc.)	
				Before	After	Before	After
		years	*mgm.*				
C. Intracutaneous							
J.O'C.	M	75	0.15	0	1 : 128	0	1,000,000
N.R.	F	78	0.1	0	1 : 4	0	100,000
J.W.	F	66	0.1	1 : 2	1 : 64	1,000	1,000,000
C.D.	M	13	0.1	0	1 : 4	0	1,000
J.O'L.	M	57	0.1	0	0	10,000	10,000
L.A.	F	49	0.1	0	0	0	0
J.C.	M	63	0.01	0	1 : 128	10	1,000,000
S.H.	F	62	0.01	0	1 : 64	0	100,000
J.C.	F	72	0.01	0	1 : 8	0	1,000,000
A.S.	F	54	0.01	0	1 : 4	0	1,000,000
H.B.	F	23	0.01	1 : 8	1 : 16	10,000	10,000,000
M.H.	F	50	0.01	0	1 : 2	0	10,000
M.M.	F	40	0.01	0	1 : 2	0	100,000
A.G.	F	38	0.01	0	0	0	10
M.W.	F	50	0.01	0	0	0	0
T.J.	M	63	0.001	1 : 2	1 : 4	10	10,000
B.H.S.	M	18	0.001	0	0	10	1,000
R.S.	M	45	0.001	0	0	100	1,000
C.D.	F	76	0.001	0	0	0	10
P.S.	M	34	0.001	0	0	0	0
D.C.	M	54	0.001	1 : 2	0	0	0
E.H.	M	45	0.0001	0	1 : 8	0	10,000
A.I.	M	32	0.0001	0	0	0	10,000
J.T.P.	M	62	0.0001	0	1 : 2	0	1,000
B.A.	M	57	0.0001	0	0	100	100
T.C.	M	66	0.0001	0	0	0	0
D.N.	M	44	0.0001	0	0	0	0
E.D.	M	68	0.0001	0	0	10	0

amounts of the carbohydrate were used there was a steady decline both in the percentage of subjects showing a response and in the amount of antibody stimulated in each subject. 3. The amount used being the same, the carbohydrate was most effective when injected intracutaneously and least when given intravenously. This was particularly true with doses of 0.1 mgm. or less. 4. The optimum response was obtained with 1.0 mgm. given subcutaneously. A similar response followed intracutaneous injections of 0.15 to 0.01 mgm. 5. The 5.0 mgm. doses failed to stimulate antibodies in most of the subjects to whom they were given intravenously, whereas 1.0 mgm. administered by the same route yielded some response in every subject. 6. Doses of 0.001 mgm. or less gave only low grade and inconsistent responses. 7. As in previous studies with other types of pneumococci (3, 4), protective antibodies were demonstrated more readily than were agglutinins.

As regards the effectiveness of the Type VIII soluble antigen given by various routes, the results of the present study in human subjects are not in accord with those which have been obtained in

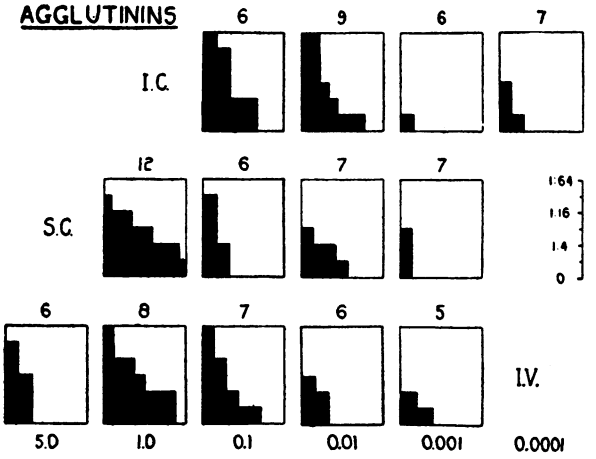

FIGURE 1

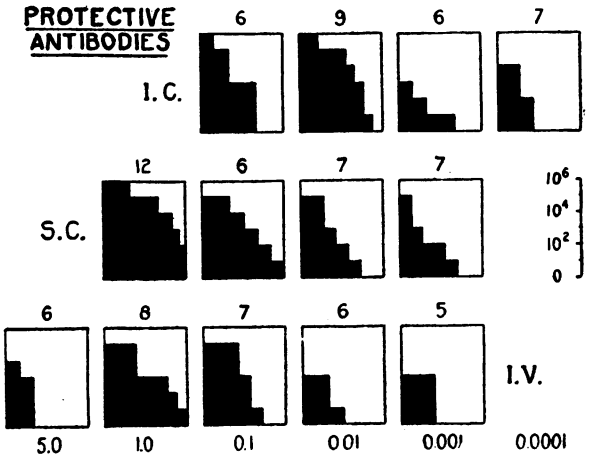

FIGURE 2

EXPLANATION OF FIGURES 1 AND 2

Each block represents the homologous type-specific antibody response of a group of patients receiving the same dose of Type VIII specific carbohydrate by the same route. The number of individuals in each group is indicated above the block. The blocks from left to right represent groups of subjects injected by the same route, viz., I.V. = intravenous, S.C. = subcutaneous and I.C. = intracutaneous. The blocks from above down represent groups which received the same dose of the carbohydrate; the amount, in milligrams, is indicated at the bottom of the figures.

The solid portions represent the increase in antibodies following the injections. The height of the solid portions represents the titer of antibody, and the width of each portion represents the percentage of subjects acquiring that titer. The scale on the right represents the maximum titer, if none was present before the injection, or the maximum increase in titer.

Figure 1 represents the agglutinin response; the scale represents serum dilutions.

Figure 2 shows the mouse protective antibody response; the scale represents fatal doses protected by 0.2 cc. of serum.

animals with the whole pneumococcus cell. Stillman (5) obtained a progressively smaller type-specific antibody response in rabbits to Type I pneumococci given by the intravenous, intraperitoneal, intramuscular, and subcutaneous routes, respectively. Furthermore, Julianelle (6) was unable to demonstrate an appreciable titer of type-specific antibodies in the sera of rabbits inoculated with Type I or Type III pneumococci intracutaneously.

Of particular interest is the failure to obtain a response, in most instances, when 5.0 mgm. were given intravenously; whereas the 1.0 mgm. injections by the same route were almost constantly effective. Such a phenomenon was noted by Schiemann and Casper (7), who found that small doses of specific precipitable substance of the pneumococcus protected mice against subsequent fatal injections of live organisms, whereas 100 times the same dose failed to protect.

In our investigation, serum was obtained for the study of antibodies in most of the subjects at weekly intervals for three or more weeks after the injection of carbohydrate. At the end of the first week only slight amounts of antibody could be demonstrated in the sera of a few individuals. This was true, in general, for each of the different amounts of material given by the various routes although some of the subjects who received intravenous injections did show relatively high titers after one week. As a rule, the maximum titers were obtained two weeks after injection. In some subjects receiving subcutaneous or intracutaneous doses, however, the titer continued to rise during the third week. In a small group of subjects whose sera were studied at intervals of two days, the increase in the titer of antibodies occurred between the seventh and ninth days, and the rise continued at a slower rate during the next five days. Similar results were obtained, in the latter experiments, both with intravenous and with subcutaneous injections.

In addition to tests for the homologous type of antibodies, all of the sera were examined for Type

III and either Type I or Type II agglutinins and, in most instances, tests for mouse protective antibodies against these types of pneumococci were carried out. Heterologous type-specific agglutinins were not demonstrated. In occasional subjects, heterologous type-specific protective antibodies were found to appear or to increase in titer in the sera obtained after the injection of Type VIII specific carbohydrate (Table II). The titers of such antibodies were always low and, with one exception, they were found to be active against the related Type III pneumococcus. In one individual, a 100-fold increase in Type I protective antibodies occurred in addition to the appearance of the Type III antibodies. Appreciable increases in phagocytic titer, without the development of demonstrable protective antibodies, were demonstrated for Type I pneumococci in two subjects and for Type II in two other individuals.

TABLE II

Development of mouse protective antibodies against heterologous types in subjects receiving injections of the specific carbohydrate of the Type VIII pneumococcus

Subject	Type VIII injection		Heterologous protective titer*		
	Route	Dose	Type	Before	After
		mgm.			
M.A.	Intravenous	5.0	III	10	100
J.B.	"	5.0	III	10	100
C.McF.	"	1.0	III	10	100
D.B.	"	1.0	III	0	10
G.M.	"	0.1	III	100	100,000
F.S.	Subcutaneous	1.0	III	0	100
W.H.M.	"	1.0	III	10	100
J.C.	Intracutaneous	0.01	III	0	100
			I	10	1,000
H.B.	"	0.01	III	100	1,000
A.S.	"	0.01	III	0	10
A.G.	"	0.01	III	0	10

* Fatal doses neutralized per 0.2 cc. serum.

For the homologous type-specific antibody response see preceding table.

SUMMARY

The specific carbohydrate of the Type VIII pneumococcus stimulates the production of homologous type-specific antibodies when given to human subjects by the intracutaneous, subcutaneous or intravenous route. The optimum dose was found to be 1.0 mgm. given subcutaneously. Smaller doses were more effective when administered intracutaneously than by the other routes. Doses of 1.0 or 0.1 mgm. were more effective when given subcutaneously than when injected intravenously. An intravenous dose of 5 mgm. failed to stimulate antibodies in most individuals, whereas 1.0 mgm. given in this manner caused a response quite constantly. A small amount of antibodies against Type III pneumococci were demonstrated in the sera of occasional subjects who had received injections of the Type VIII carbohydrate.

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