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Naturally occurring antibodies to *Neisseria meningitidis*

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Immunity in meningococcal diseases has been inadequately studied and, until now, most attempts at serotherapy and immunization against meningococcal meningitis have been unsuccessful. To ascertain the status of immunity among children at greatest risk of contracting the disease, 267 serum specimens from healthy children aged from 1 month to 5 years were examined with Farr's radioimmunological technique, as simplified by Gotschlich. The results showed that the children must have received maternal antibodies through the transplacental route. Antibodies persisted up to the third month of life, after which they were virtually undetectable until, at about 8 months of age, antibodies reappeared. Subsequently there was a gradual rise, especially from 2 to 5 years, 97 % of children in the highest age group having serum antibody levels of 0.479 μg/ml or more. These children may have had meningococcal infections that were wrongly diagnosed as respiratory disease of unknown etiology.

Immunity in meningococcal diseases has been studied intensively during epidemics, but hardly at all between epidemics. Because the subject has not received the attention it deserves, many problems concerning the antigenicity, pathogenicity, and epidemiology of meningococcal infections remain unsolved. Before the era of chemotherapy, most studies in that field dealt with the classification of *Neisseria meningitidis*. Several classifications were elaborated and a unified system was established in 1958, when four serological groups—A, B, C, and D—were internationally recognized. In the sixties, further serological groups were discovered and provisionally designated as X, Y, Z, 29E, and W135.

The first attempts at serotherapy of meningococcal meningitis and at immunization against the disease were made in the thirties. Most of the attempts failed because at that time there was insufficient knowledge of *N. meningitidis* serological groups. After the advent of chemotherapy, interest in vaccination died down until the sixties, when the resistance of *N. meningitidis* to sulfonamides became evident. Since then, several groups of workers have been trying to prepare an effective vaccine. One such group, led by one of us (E.G.), has developed a promising vaccine by isolating polysaccharide from the walls of *N. meningitidis* cells of serological groups A and C.

The object of the present study was to acquire preliminary information on the state of immunity in the child population at greatest risk from the most feared form of meningococcal infection—cerebrospinal meningitis. The aim was to collect data with a view to effective prevention by means of immunization and to ascertain the age limit for vaccination. Data on the reactivity and antibody response of the polysaccharide vaccine against *N. meningitidis* will be published once they have been statistically analysed.

MATERIALS AND METHODS

Collection of sera

Sera were collected from healthy children who had not received immunoglobulin or a blood transfusion during the few weeks before the collection of blood samples. Two collections were made: (A) 170 serum specimens were selected from those obtained in a multipurpose collection from children aged 2–5 years. The sera were divided into aliquots and stored

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Table 1. Distribution of antibody level in children 0–5 years of age, according to age groups

Antibody levels (μg/ml)		Age (months)																			
		1	2	3	4	5	6	7	8	9	10	11	12-14	15–17	18–20	21–23	24–35	36–47	48–59	60–71	Total
< 0.275		1	4	10	10	5	5	4	4	2	2	2	2	1	1						54
0.276–0.330			1					1					2			2	4	1	1	1	13
0.331–0.397								1				2	1	2		2	3	1	1		13
0.398–0.478										1	1	2	1	1			4		3		13
0.479–0.574			1							1		1	1				2	2	2		10
0.575–0.691		2											2				5	4	1	1	15
0.692–0.831		1											1	1	2		3	1	1		10
0.832–0.999		2	1											1			1				5
1.000–1.201			1													2	10	5	6	5	29
1.202–1.444																	12	8	10	3	33
1.445–1.737																	5	4	6	4	19
1.738–2.089																	7	2	5	7	21
2.090–2.511																		1	5	4	10
2.512–3.019																		2	1	1	4
3.020–3.630																	1	1	1	2	5
3.631–4.364																	1	1	2	2	6
4.365–5.248																			1	3	4
5.249–6.309																	1		1	1	3
Total		6	8	10	11	5	5	6	4	4	3	7	10	6	3	6	59	33	47	34	267
⩾ 0.479	No.	5	3							1		1	4	2	2	2	48	31	42	33	174
	%	83	38							25		14	40	33	67	33	81	94	89	97	65
⩾ 1.000	No.		1													2	37	24	38	32	134
	%		12													33	63	73	81	94	50

at −20°C at the WHO Reference Serum Bank in Prague until used, in October 1971. (B) In November 1971, 97 serum specimens were collected from healthy children aged 1–23 months in four nurseries and one children's home in Bohemia. The sera were divided into aliquots and stored in the freezer until used, in December 1971. Both collections of sera were airmailed on dry ice to E.G.'s laboratory at the Rockefeller University, New York. The sera reached their destination within 15 hours of despatch.

Nasopharyngeal swabs

Nasopharyngeal swabs were made in all children of collection group B before blood samples were taken. The swabs were immediately inoculated on to Thayer–Martin medium, placed in a thermobox heated to 37°C, and transported to our laboratory. After incubation at 37°C in a CO_2 atmosphere, the results were read at 24 h and 48 h. The strains isolated on Mueller–Hinton medium were identified according to their growth properties, microscopic picture, oxidase reaction, and biochemical characteristics. The final identification was made by serological methods. *N. meningitidis* was identified by means of the standard methods of the WHO International Reference Laboratory for Meningococci, Marseilles, which we supplemented with further criteria for differential diagnosis.

Serological examinations

Farr's radioimmunological technique with Gotschlich's simplified modification was used. The externally labelled polysaccharide isolated from the walls of *N. meningitidis* group A cells served as a radioisotope-labelled antigen. Radioactive iodine-125 and sodium-22 were used as volume markers.

A mixture of 10 μl of labelled antigen and 10 μl of inactivated serum was prepared and left in the refrigerator at 4°C overnight. The next day, 20 μl of 80% ammonium sulfate were added, thus provoking precipitation of the antigen–antibody complex. After centrifugation, the supernatant fluid was removed with a vacuum pump. The precipitated sediment was examined with a double-channel Gamma-Spectrometer (Nuclear, Chicago, model 4233). The results were compared with the known standard, and the weight of antibodies per millilitre of serum was calculated in micrograms.

RESULTS

The antibody levels against *N. meningitidis* group A polysaccharide, for the 267 sera examined, estimated in μg/ml of the serum, are shown in Table 1.

Antibody levels were classified according to age, in geometrical series of dilutions: 0.275 μg/ml–6.309 μg/ml. The numbers of children in the various age groups are given in the lower part of the table. Excepting children in the first two age groups (1 and 2 months of age), in whom transplacental maternal antibodies persisted up to the third month of life, virtually no antibodies were detectable until the age of 8 months. Subsequently a gradual rise in the antibody level was observed up to the age of 24 months, when the rise became more marked up to 5 years of age. Most of the children in the last four age groups possessed high antibody levels.

Owing to the lack of data on children with cerebrospinal meningococcal meningitis (CSM), it is still not known what antibody level ensures immunity. However, on the basis of Table 1, we established the minimum protective level as 0.479 μg/ml and the safe protective level as 1.0 μg/ml or more. The frequency of antibody titres higher than the two limiting values is shown in the bottom two rows of the table. Of the 6, 8, and 10 children aged 1, 2, and 3 months, respectively, Table 1 indicates that 5 (83%) of the infants aged 1 month and 3 (38%) of those aged 2 months were protected, maternal antibodies being completely eliminated by the third month of life. The ages of the mothers ranged from 16 to 27 years.

The differences in the mean values of antibody titres between children living in families and those living in crèches or nursery schools are shown in Table 2. The statistical evaluation was based on Student's *t* test and on the presumption of a loga-

Table 2. Differences in mean values of antibody titres between children living in families and those living in crèches or nursery schools

Age (years)	Environment	Geometric mean of titres	*n*	*t*
2	family	0.971	21	0.46
	crèche	0.884	24	
3	family	1.661	7	2.06
	nursery school	0.936	18	
4	family	1.577	6	0.58
	nursery school	1.342	37	
5	family	1.639	6	0.75
	nursery school	2.026	25	

Table 3. Comparison of antibody levels of children of different age groups

Age compared (years)	Geometric mean value of titres	P	Arithmetic mean value of titres	P
2 and 3	0.8702		0.08716	
2 and 4	1.9512	0.05	0.78622	
2 and 5	3.8335	< 0.01	2.05580	< 0.05
3 and 4	0.9432		0.80615	
3 and 5	2.8010	< 0.01	2.15668	< 0.05
4 and 5	2.0342	< 0.05	1.59392	

rithmic-normal distribution of titres. Since the *t* test values were not statistically significant, it is also justifiable to consider the differences between antibody titre means as not statistically significant.

In the absence of a statistically significant difference between the antibody levels of children living in families and those in a collective environment, a comparison of antibody titres of separate age groups of the study population was made by means of Student's *t* test. The results are given in Table 3. On the one hand, the test was applied to the difference of arithmetic means (under the assumption of a normal distribution of antibody titres) and, on the other hand, to the difference of geometric means (under the assumption of a logarithmic-normal distribution of antibody titres).

The difference between the age groups compared was considered to be statistically significant if the *t* test value, under the assumption of a zero hypothesis, had a probability of less than 0.05 (or 0.01). Thus a statistically significant difference was found between children aged 2 and 5 years, 3 and 5 years (comparing both arithmetic and geometric means), and 4 and 5 years (comparing geometric means).

Bacteriological examination

Bacteriological examination of the 97 sera collected from healthy children yielded 3 *N. meningitidis* strains that could not be typed. The strains met all the criteria for *N. meningitidis*, but on serological examination did not agglutinate with any of the known antimeningococcus sera used in Czechoslovakia at the time of the study (A, B, C, D, X, Y, and Z). Two of the strains were isolated from two boys, one aged 14 months (with an antibody level of 0.51 μg/ml) and the other 48 months (with an antibody level of 0.26 μg/ml). The third strain was isolated from a 14-month-old girl with an antibody level of 0.61 μg/ml.

In addition, *N. lactamica* was isolated from four boys aged 7, 14, 22, and 24 months.

DISCUSSION

The aim of the study was to gain a preliminary idea of the antibody levels in children against the polysaccharide of group A *N. meningitidis*. The study was carried out before verification of the antibody reaction following the administration of polysaccharide vaccine against group A *N. meningitidis*. The results show that the most suitable age for primary vaccination is 3–10 months, when children do not possess any detectable antibodies. We believe that, in such children, it might be possible to evaluate the immunogenicity of the vaccine since, with later immunization, when active antibodies are already present, a booster effect appears to take place. That booster effect, in our experience, may be provoked by a very small dose of the antigen, which may elicit considerable antibody response.

In some children over 24 months of age, very high antibody levels were found—comparable to, or even higher than, those in adults. These findings led us to examine the case histories of the children concerned. Most of them had repeatedly suffered from rhinopharyngitis, bronchitis, tracheobronchitis and, in some cases, even tracheitis and laryngotracheitis.

It has long been known, in Czechoslovakia, that *N. meningitidis* may be an etiological agent of respiratory diseases, but little attention has been paid to this fact so far. The clinician thinks of a meningococcal etiology only in cases of the cerebrospinal meningitis syndrome when he makes a bacteriological examination of clinical material. Respiratory diseases are usually attributed to streptococci, *Haemophilus influenzae*, *Bordetella pertussis*, and *B. parapertussis*. Bacteriological examinations are conducted accordingly, negative culture results being

considered as evidence of a viral etiology. The possibility of *N. meningitidis* is thus usually excluded from routine examinations. Only in recent years has attention slowly turned again to this possibility, and we have actually found several reports on the role of this organism in respiratory diseases. That is one of the reasons why we subject material from patients with such diseases to bacteriological examination. The results of that investigation will be published later.

The diagnosis of upper respiratory tract diseases should be thoroughly revised and examinations made more detailed, so as to clarify the etiology of such diseases.

High antibody levels in children aged 2 years or more may indicate that these children have survived meningococcal infections wrongly diagnosed as respiratory disease of unknown etiology. Corroboration of this theory by the detailed examination of a greater number of children would provide valuable data on the epidemiology of meningococcal infection.

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RÉSUMÉ

ANTICORPS NATURELLEMENT PRÉSENTS CONTRE *NEISSERIA MENINGITIDIS*

Pour apprécier le degré d'immunité dont bénéficient des enfants considérés comme particulièrement exposés au risque de contracter une méningite cérébro-spinale à méningocoques, 267 échantillons de sang prélevés sur des enfants en bonne santé de 1 mois à 5 ans ont été examinés en recourant à la technique radioimmunologique de Farr, simplifiée ensuite par Gotschlich. Pour déterminer les titres d'anticorps par μg/ml de sérum, le polysaccharide isolé sur la membrane externe de *Neisseria meningitidis* du groupe A a été marqué extrinséquement au moyen d'iodine-125 radioactive.

On a déduit des résultats obtenus que les enfants devaient avoir acquis des anticorps maternels par voie transplacentaire. Ces anticorps persistent jusqu'au troisième mois de la vie, après quoi ils deviennent pratiquement impossibles à déceler jusqu'à l'âge de huit mois environ. On constate ensuite une élévation progressive de leur taux, surtout dans les groupes d'âge 2–5 ans, 97% des enfants de 5 ans (60–71 mois) présentent un titre d'anticorps égal ou supérieur à 0,479 μg/ml. Les auteurs pensent qu'il est possible que ces derniers aient été atteints d'infections méningococciques faussement diagnostiquées comme maladies respiratoires d'étiologie inconnue.