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The Content of Antigens 1, 2 and 3 in Strains of *Bordetella pertussis* and in Vaccines

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ABSTRACT

A comparison was made of the content of heat-labile antigens 1, 2 and 3 in strains of *Bordetella pertussis* isolated in Toronto, and the content of the same antigens in vaccines being used in that community. Antisera were prepared in rabbits and made monospecific by absorptions. Antigens were detected by slide agglutination tests. Of the 58 strains examined, 56 contained antigens 1 and 3 but no antigen 2 was detected. Nine of these strains were isolated from children who had received a full course of vaccination and had subsequently developed pertussis. All 10 vaccines examined contained antigen 1; two, possibly four, contained no antigen 2; and seven of the 10 vaccines were weak or deficient in antigen 3. This work suggests that improved protection might be attained by the use of vaccines containing adequate amounts of all three antigens.

SOMMAIRE

On a comparé la teneur en antigènes thermo-labiles 1, 2 et 3 de souches de *Bordetella pertussis* isolées à Toronto et la teneur dans les mêmes antigènes de vaccins employés dans cette collectivité. Les antisérums préparés sur des lapins ont été rendus monospécifiques par absorption. On a décelé les antigènes par les épreuves d'agglutination sur lames. Sur les 58 souches étudiées, 56 contenaient les antigènes 1 et 3 mais aucune ne contenait l'antigène 2. Neuf de ces souches avaient été isolées chez des enfants qui avaient eu une vaccination complète et qui avaient contracté la coqueluche ultérieurement. Tous les 10 vaccins examinés contenaient l'antigène 1; deux, peut-être quatre vaccins ne contenaient pas l'antigène 2, et sept des 10 vaccins avaient une teneur faible en antigène 3 ou n'en contenaient pas. Ce travail permet de croire qu'on peut s'assurer une meilleure protection par l'emploi de vaccins qui contiennent des quantités adéquates des trois antigènes.

IN CANADA, the use in vaccines of the combined antigens diphtheria-pertussis (DP) was adopted generally in 1943 and those of diphtheria-pertussis-tetanus (DPT) in 1948.[1] A series of field trials with pertussis vaccines was carried out in the United Kingdom between 1942 and 1957.[2] Massachusetts introduced general vaccination against whooping cough in 1949,[3] and in 1953 the Netherlands began a program of mass pertussis immunization.[4] Reports of greatly diminished attack and mortality rates for whooping cough emerged from these studies, together with observations of diminished severity in vaccinated children who contracted the disease. Periodically since then, doubt has been cast on the effectiveness of particular vaccines. Edsall *et al.*[5] in 1962 found that the pertussis component of some batches of "quadruple antigen" did not meet the potency requirements of the National Institutes of Health, Bethesda, Md. Preston,[6] in Britain, in 1963 suggested that deficiencies in heat-labile antigens in pertussis vaccine may result in failure to give complete protection, and Olson, Eldering and Graham[7] in 1964 found that the presence of benzethonium chloride as a preservative in pertussis vaccine greatly diminished its potency. Whatever the reason, some children who have had a full course of pertussis vaccination, including a booster dose, develop whooping cough.

The complexity of the antigenic structure of *Bordetella pertussis* has been well recognized since Andersen,[8] in 1953, reported the results of her serological studies. Since then, further information has been forthcoming from the work of Eldering and her co-workers,[9, 10] and from other investigators.[11-15] The subject of *Bord. pertussis* antigens was recently reviewed by Munoz.[16]

The present report deals with a comparison of the content of the major heat-labile antigens 1, 2 and 3 (but not antigen 5 or the minor antigen 4) in strains of *Bord. pertussis* isolated in Ontario during the past few years and the content of the same antigens in vaccines currently being used in Canada. The majority of strains examined were isolated in the bacteriology department of The Hospital for Sick Children, Toronto. Most of the patients came from Toronto and the remainder from neighbouring counties.

MATERIALS AND METHODS

Cultures.—Cultures of *Bord. pertussis* 353Z (heat-labile antigen 1, plus heat-stable antigens),

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M2 (heat-labile antigens 1 and 3 plus heat-stable antigens), 3747 (heat-labile antigens 1, 2 and 3 plus heat-stable antigens) for the production of antisera were kindly provided by Dr. N. W. Preston, Department of Bacteriology, University of Manchester. In addition to the strains from patients of The Hospital for Sick Children, four further strains were provided by Dr. C. R. Amies, Chief Bacteriologist, Ontario Department of Health Central Laboratory.

Production of antisera.—Antisera were produced in young adult white rabbits with no agglutinating antibodies to strains 353Z, M2 or 3747 at a titre of 1:4 or more prior to vaccination. Four rabbits were used with each strain. A series of six intravenous injections of formol-killed organisms grown in modified Hornibrook medium (provided by Connaught Medical Research Laboratories, Toronto) were administered to each rabbit over a period of 17 days and totalled approximately 90,000 million organisms. After it had been confirmed that the antibody titres had risen sufficiently, the rabbits were bled two weeks after the last injection.

Absorptions.—The three antisera were diluted 1:2.5 in phosphate-buffered saline (PBS) at pH 7.3. Emulsions of the three strains of *Bord. pertussis* in PBS were autoclaved at 120° C. for one hour. Each serum was absorbed with a centrifuged deposit of its homologous strain by shaking for 15 minutes at room temperature in a test tube on a Vortex mixer. The organisms were then separated from the antiserum by centrifugation. Absorptions were repeated till the antisera no longer agglutinated autoclaved homologous organisms. An average of two absorptions was necessary. This method of absorption was used by Preston.[6] This serum was then considered specific for antigen 1. To make the remaining two antisera monospecific for antigens 2 and 3, anti-3747 antiserum was absorbed with strain M2 and anti-M2 antiserum was absorbed with strain 353Z. The antisera were diluted further to 1:10 in PBS. Centrifuged deposits of live heterologous strains of *Bord. pertussis* were prepared and shaken with the appropriate antiserum on the Vortex mixer for 10 minutes at room temperature. Thereafter the mixture of organisms and antisera was placed in a water bath at 37° C. for four hours with periodic inversion to keep the organisms suspended. The organisms were then separated from the antiserum as before and agglutination tests were done to prove the antiserum monospecific. Again an average of two absorptions was necessary. This method of absorption is a combination of the method described by Preston and Te Punga[17] in 1959 and that described by Eldering, Hornbeck and Baker[9] in 1957, with the modification that we found it necessary to use live organisms. Agglutination tests were done according to the method of Preston and Te Punga.[17]

Monospecific antisera.—These were used at four times the strength of the highest dilution giving strong agglutination with a homologous strain, i.e. when the end-point of strong agglutination was reached at a dilution of 1 in 400; antiserum was then used for testing cultures and vaccines at a dilution of 1 in 100. The titres of fully absorbed sera used varied from 1 in 80 to 1 in 640.

Serological typing by slide agglutination tests.—Strains of *Bord. pertussis* for typing were grown on Bordet-Gengou medium, emulsified in PBS (pH 7.3) in a test tube and shaken on a Vortex mixer to make a uniform suspension of opacity roughly equivalent to a No. 10 Brown's opacity tube. The vaccines tested contained either 15,000 million or 40,000 million organisms/ml. Four tests were done simultaneously on a glass slide, using four drops of bacterial suspension and one drop each of monospecific antisera 1, 2 and 3 and a drop of preimmunization rabbit serum diluted with PBS as a control. The slides were rocked gently in a moist chamber to prevent drying, and readings were made after five minutes. It is perhaps worth noting that strains grown on Lacey medium were found unsuitable for agglutination tests, as it was often impossible to obtain a smooth emulsion. This may have been due to the chemical differences between the two media and a consequent change in the bacterial cell.

RESULTS

The results of typing strains of *Bord. pertussis* isolated from patients between August 1963 and September 1964 are given in Table I.

TABLE I.—CONTENT OF ANTIGENS 1, 2 AND 3 IN STRAINS OF *Bord. pertussis* ISOLATED BETWEEN AUGUST 1963 AND SEPTEMBER 1964

Antigen 1 only	0
Antigens 1 and 2 only	1
Antigens 1, 2 and 3	1
Antigens 1 and 3 only	56
Total number of strains tested	58

It will be seen that 56 of 58 strains isolated from patients during the past year contained antigens 1 and 3 only. One strain contained all three antigens and one contained only antigens 1 and 2. All strains contained antigen 1. Four strains isolated in Ontario between 1959 and 1961 were examined. All strains contained antigens 1 and 3 only.

Table II gives the results of testing 10 Canadian pertussis or pertussis-containing vaccines within their expiry date. There were differences in antigenic content of the vaccines from different manufacturers and also in different batches from the same firm. Vaccines containing alum phosphate were technically difficult to test. Strong agglutination reactions were obtained with antiserum to antigen 1, but little reliance could be placed on the results of agglutination tests done with antisera

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TABLE II.—CONTENT OF ANTIGENS 1, 2 AND 3 IN 10 CANADIAN PERTUSSIS OR PERTUSSIS-CONTAINING VACCINES

Manufacturer	*Type of vaccine*	*Antigen 1*	*Antigen 2*	*Antigen 3*
A	DPT Lot 1	Strong reaction	Strong reaction	Strong reaction
	DPT Lot 2	" "	" "	Weak "
	DPT Polio Lot 1	" "	" "	Strong "
	DPT Polio Lot 2	" "	" "	" "
	Pertussis Lot 1	" "	" "	Weak "
	Pertussis Lot 2	" "	" "	Very weak or absent reaction
B	DTP (Alum PO_4) Lot 1	" "	Doubtful "	Doubtful "
	DTP (Alum PO_4) Lot 2	" "	" "	" "
	DTP combined Lot 1	" "	No "	No "
	DTP combined Lot 2	" "	" "	" "

2 and 3. All vaccines tested gave strong reactions with factor 1 antiserum. Manufacturer A had adequate amounts of antigen 2 in all six vaccines but in three vaccines had only small amounts of antigen 3. Manufacturer B in two, possibly four, vaccines had no detectable antigens 2 or 3.

Of the 58 patients, nine (16%) had had a full course of immunization against whooping cough including, in older children, a booster dose. Table III shows the antigen content of the strains isolated from these nine children. All strains contained antigens 1 and 3, and no strain reacted with antiserum 2.

TABLE III.—CONTENT OF ANTIGENS 1, 2 AND 3 IN NINE STRAINS OF *Bord. pertussis* ISOLATED FROM FULLY IMMUNIZED CHILDREN BETWEEN AUGUST 1963 AND SEPTEMBER 1964

Antigen	*1*	*2*	*3*
Number of strains reacting...	9	0	9

DISCUSSION

Eldering, Eveland and Kendrick[10] suggested that antigen 1 is the protective antigen. The content of antigens 2 and 3 varied from vaccine to vaccine. Nearly one-sixth of the children with pertussis in this series had had a full course of immunization, probably with vaccines also rich in antigen 1, since, according to Eldering, Eveland and Kendrick,[10] all strains of *Bord. pertussis* contain antigent 1. Preston and Evans[18] have produced evidence that antigens 1, 2 and 3 are all concerned with protection in mice and suggest that they may also be concerned with protection in children. Examination of vaccines being used currently in Ontario reveals that although vaccines generally are rich in antigen 1, those from manufacturer A are sometimes weak in antigen 3 and two of those from manufacturer B are deficient and two doubtfully deficient in both antigens 2 and 3.

Several possible reasons might account for the failure of vaccines to exert a protective effect. The child may fail to produce an adequate antibody response; the potency of the vaccine may have diminished, owing to incorrect or protracted storage or to the incorporation of inappropriate preservatives; an adequate response may have been produced but antibody levels may have fallen; the strain causing the infection may be particularly "virulent" or the antigenic composition of the vaccine used is not the same as that of the infecting strain. In view of the fact that the prevalent strain of *Bord. pertussis* in Ontario contains antigens 1 and 3 and that all nine cases of whooping cough in vaccinated children were caused by such strains, it would seem advisable that vaccines contain adequate amounts of antigen 3 in addition to antigen 1. As antigens 1, 2 and 3 are probably all protective, the vaccine should contain antigen 2 as well. This work suggests that had all vaccines consistently contained antigen 3, the number of vaccinated children subsequently developing whooping cough might have been lower.

SUMMARY

The content of heat-labile antigens 1, 2 and 3 was examined in 58 strains of *Bord. pertussis* isolated from patients of The Hospital for Sick Children, Toronto, during the past year. Fifty-six of 58 contained only antigens 1 and 3; one contained in addition antigen 2; and one only antigens 1 and 2. Nine of the strains examined were isolated from children who had been fully vaccinated against pertussis; all of these strains contained antigens 1 and 3. Vaccines being used at the present time in Toronto were also examined. They were all found to contain large amounts of antigen 1; two, possibly four, failed to contain antigen 2; seven of 10 vaccines were weak or deficient in antigen 3. It is possible that increased protection might be attained through the use of vaccines containing adequate amounts of all heat-labile antigens 1, 2 and 3.

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REFERENCES

1. WILSON, R. J.: *Canad. J. Public Health*, 53: 457, 1962.
2. Great Britain Medical Research Council. Whooping Cough Immunization Committee: *Brit. Med. J.*, 1: 994, 1959.
3. PROVENZANO, R. W., WETTERLOW, L. H. AND IPSEN, J.: *New Eng. J. Med.*, 261: 473, 1959.
4. COHEN, H. H.: *Antonie Leeuwenhoek*, 29: 183, 1963.
5. EDSALL, G. *et al.*: *New Eng. J. Med.*, 267: 687, 1962.
6. PRESTON, N. W.: *Brit. Med. J.*, 2: 724, 1963.
7. OLSON, B. H., ELDERING, G. AND GRAHAM, B.: *J. Bact.*, 87: 543, 1964.
8. ANDERSEN, E. K.: *Acta Path. Microbiol. Scand.*, 33: 202, 1953.
9. ELDERING, G., HORNBECK, C. AND BAKER, J.: *J. Bact.*, 74: 133, 1957.
10. ELDERING, G., EVELAND, W. C. AND KENDRICK, P. L.: *Ibid.*, 83: 745, 1962.
11. PILLEMER, L., BLUM, L. AND LEPOW, I. H.: *Lancet*, 1: 1257, 1954.
12. BANERJEA, A. AND MUNOZ, J.: *J. Bact.*, 84: 269, 1962.
13. LIN, T. M.: *J. Formosa Med. Ass.*, 62: 226, 1963.
14. BARTA, G.: *Canad. J. Microbiol.*, 9: 629, 1963.
15. SUTHERLAND, I. W.: *Immunology*, 6: 246, 1963.
16. MUNOZ, J. J.: *Bact. Rev.*, 27: 325, 1963.
17. PRESTON, N. W. AND TE PUNGA, W. A.: *J. Path. Bact.*, 78: 209, 1959.
18. PRESTON, N. W. AND EVANS, P.: *Nature (London)*, 197: 508, 1963.