Papers and Originals

Diagnosis of Whooping Cough : Comparison of Serological Tests with Isolation of Bordetella Pertussis

A Combined Scottish Study*

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Summary: Antibody titres for *Bordetella pertussis* in paired sera from 223 children with suspected whooping cough showed good correlation by complement fixation and agglutination techniques. The patient's age was related to serological responses and in a different way to *B. pertussis* isolations: in 73 children under 6 months of age *B. pertussis* was isolated from nasopharyngeal secretions in 42% and serological findings were positive in 19%; in 11 patients over 1 year of age serological tests were positive in 65% and cultures in 19%.

***B. pertussis* was isolated from 59 out of 210 patients, while rising antibody titres were found in a further 43 from whom no isolations were made; thus 102 (49%) out of 210 showed evidence of infection with *B. pertussis*. Serotype 1,3 was the commonest serotype isolated from both immunized and unimmunized children. Previous immunization appeared to reduce the chances of isolating *B. pertussis*.**

Introduction

The appearance of specific complement-fixing antibodies in the blood after infection with *Bordetella pertussis* was first reported by Bordet and Gengou (1906, 1907); this was confirmed between 1915 and 1938 by many other workers (cited by Wilson and Miles, 1964). Donald (1938) compared complement fixation and agglutination tests in the diagnosis of whooping cough and claimed that complement fixation tests were more reliable than agglutination tests. On the other hand, Evans and Maitland (1939a, 1939b) found that agglutination was more sensitive than the complement fixation technique for detecting specific *B. pertussis* antibodies, and since 1940 agglutination has been the technique mainly used for detecting these specific antibodies. Recently, however, the need for standardizing all reagents used in complement fixation tests has been emphasized and the complement fixation technique has become more reliable and sensitive (Bradstreet and Taylor, 1962; Casey, 1965; Grist *et al.*, 1966). Since complement fixation is now the main technique used for serological diagnosis of other respiratory infections the opportunity was taken to reassess its value in the diagnosis of whooping cough, and also to compare serological results with *B. pertussis* isolations, during a combined Scottish study of clinical, bacteriological, and virological findings in children with suspected whooping cough from 1966 to 1968. Correlation of clinical and laboratory findings will be reported at a later date.

Materials and Methods

Serological Tests.—Paired sera were received from 223 children in Dundee, Edinburgh, and Glasgow in whom a clinical diagnosis of whooping cough was sustained throughout the illness. The first specimen of serum was collected shortly after the child's admission to hospital and the second usually 14 days later. Sera were examined by both complement fixation and agglutination tests, which were carried out independently in separate laboratories—the complement fixation test in the Regional Virus Laboratory at Ruchill Hospital, Glasgow, and the agglutination tests at the Bacteriology Laboratory, City Hospital, Edinburgh.

All titres throughout this paper are expressed as reciprocals. Titres were considered to be rising if a fourfold or greater difference between acute and convalescent sera was obtained; this includes complement fixation titres rising from <8 (the lowest dilution) to 16, and agglutination titres from <15 (the lowest dilution) to 30. High titres comprise titres in one or both sera $\geqslant 64$ by complement fixation or $\geqslant 60$ by agglutination tests.

Agglutination Tests.—Each batch of antigen was prepared from a freshly isolated culture of *B. pertussis* serotype 1,3. Several batches of antigen were required and different strains were used for each batch. The growth was emulsified in saline and the suspension, without further treatment, was diluted until it contained 10^9 organisms per ml. by Brown's opacity tubes. The suspension was added in 0·4-ml. volumes to 0·4 ml. of doubling dilutions in saline of patients' serum. The mixtures were incubated for 18 hours in a water-bath at 37°C. Readings were made by eye-glass magnification, endpoints being taken as the highest serum dilution showing complete agglutination.

Complement Fixation Tests.—The antigen used was prepared from a strain of *B. pertussis* serotype 1,3 isolated from pharyngeal secretions of one of the children in the study; it was a different strain from those used for the agglutination tests. The method used was a modification of that described by Price (1932) for preparing a gonococcal complement fixation antigen. The organism was cultured aerobically for three days at 36°C. on several 8·5-cm. diameter plates of Bordet-Gengou medium (Cruickshank, 1965). The growth was washed off with normal saline, using 5 ml. per plate, and filtered through sterile gauze to remove fragments of medium; 0·2 ml. N/1 sodium hydroxide was added per 5 ml. suspension, which was then placed in a water-bath at 37°C. for two hours and the pH was adjusted to 7·4 with N/1 hydrochloric acid. The preparation was then heated to 56°C. for 30

*This study was an extension into Scotland of the Public Health Laboratory Service Working Party's investigation on the efficacy of whooping cough vaccines in the United Kingdom before 1968 (*British Medical Journal*, 1969, **4**, 329). Professor R. Cruickshank, the original convener of the Scottish Group, was the prime mover in the Scottish Study, which was supported by the Scottish Home and Health Department.

The participants in this study were: Dr. Margaret C. Calder, Bacteriology Laboratory, City Hospital, Edinburgh (secretary); Professor R. Cruickshank, Bacteriology Department, University of Edinburgh (convener until September, 1968); Professor J. P. Duguid, University of Dundee; Dr. T. F. Elias-Jones, City Laboratory, Glasgow; Dr. R. J. Fallon, Department of Pathology, Ruchill Hospital, Glasgow, N.W.; Dr. R. R. Gillies, Bacteriology Department, University of Edinburgh (convener since September, 1968); Dr. D. M. Green, Bacteriology Department, University of Dundee; Professor N. R. Grist, Ruchill Hospital, Glasgow, N.W.; Dr. W. M. Jamieson, King's Cross Hospital, Dundee; Dr. J. H. Lawsen, Ruchill Hospital, Glasgow; Dr. W. C. Love, Belvidere Hospital, Glasgow; Dr. P. McKenzie, Belvidere Hospital, Glasgow; Dr. I. W. Pinkerton, Ruchill Hospital, Glasgow, N.W.; Dr. Constance A. C. Ross, Virus Laboratory, Ruchill Hospital, Glasgow, N.W.; Dr. G. Sangster, City Hospital, Edinburgh; Dr. A. M. M. Wilson, Bacteriology Department, University of Edinburgh.

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minutes to reduce anticomplementary activity, and sodium azide added to a concentration of 0·08%. The optimal dilution of each batch of antigen was determined by chessboard titration against pooled sera from children under 1 year old convalescing from *B. pertussis* infection. The antigen was stored at 4°C.; it remained stable for at least six months. Complement fixation tests on sera were carried out as described by Grist *et al.* (1966), except that a microtitre technique was used, the unit volume of each reagent being 0·025 ml.

B. pertussis Isolations.—Pernasal swabs (at least three from each patient at daily intervals from admission to hospital) from 210 of the 223 patients were inoculated either directly on to suitable culture medium or placed in a carrier medium for inoculation next day. Carrier media were as previously described (Public Health Laboratory Service Report, 1969). Culture media comprised Bordet-Gengou medium and/or charcoal blood agar; after inoculation culture plates were incubated aerobically at 37°C. for five to seven days. *B pertussis* was identified by standard bacteriological methods and typed as described by Preston (1965) with monospecific sera prepared by Wellcome Research Laboratories to antigens 1,2 and 3.

Results

Comparison of Antibody Response (Table I).—Rising antibody titres were obtained by both complement fixation and agglutination techniques in 39 patients, by complement fixation only in 18, and by agglutination only in nine. Agreement between the two tests was measured by co-positivity and co-negativity as defined by Buck and Gart (1966). Thus, taking positive serological findings as comprising rising titres or high titres and negative findings as low or undetectable titres, the co-positivity of complement fixation compared with agglutination as the standard technique was 70 (89%) of 79, and the co-negativity 128 (89%) of 144; overall agreement 198 (89%) of 223—that is, there was good correlation between the tests.

B. pertussis Isolation and Rising Antibody Titres (Table II).—For this comparison rising titres only have been considered, since high but not rising titres might be due to past infection or previous immunization. Of the 210 patients from whom specimens of paired sera and pernasal swabs were received, 59 (28%) yielded *B. pertussis* (48 type 1,3; 8 type 1,2,3, and 3 untyped); 21 (36%) of these 59 showed rising titres by complement fixation and/or agglutination: 12 by both tests, 7 by complement fixation alone, and 2 by agglutination alone. From three of these 21 patients with positive cultures and rising titres (three by complement fixation, two by agglutination) the serotype isolated was 1,2,3; this indicated that our complement fixation antigen detected antibody responses not only to serotype 1,3 but also to serotype 1,2,3. Of the 151 patients from whom *B. pertussis* was not isolated, 43 (28%) showed rising titres. Positive cultures with no rising titres by either complement fixation or agglutination were found in 38 patients; in 35 of these 38 antibodies were either undetectable or of low titre.

Age of Patient.—The number and percentage of patients in various age groups with positive cultures, positive serology (rising or high titres), and positive cultures and/or serology are given in Table III. There were 73 (35%) patients under 6 months of age; in these a much higher proportion yielded *B. pertussis* (42%) than positive serology (19%). In contrast, each of the three age groups over 1 year old showed higher figures for positive serology than for positive cultures. Of the 111 patients over one year of age *B. pertussis* was isolated from 21 (19%), and a positive serological response was obtained in 72 (65%).

Previous Immunization.—Details of previous immunization against *B. pertussis*, whether full, partial, or nil, were obtained in 91 patients with definite evidence of *B. pertussis* infection by isolation and/or rising antibody titre; 25 (27%) of these 91 had received full immunization and 62 (68%) had had no previous immunization (Table IV). *B. pertussis* was isolated from seven (28%) of those fully immunized (serotype 1,3 from six) compared with 45 (73%) of the unimmunized (serotype 1,3 from 39); the difference is highly significant ($P<0.001$), suggesting that previous immunization suppressed growth of *B. pertussis* in the nasopharynx. In contrast those fully immunized showed a higher proportion with rising antibody titres—24 (96%) compared with 27 (44%) of the unimmunized ($P<0.001$). Age, however, was another factor which might have been related to this difference in serological response, as only five (20%) of the fully immunized were under 1 year old compared with 38 (61%) of the unimmunized (Table V).

Discussion

In the present study *B. pertussis* antibody titres showed good correlation by complement fixation and the previously accepted agglutination technique. Also in patients in whom

TABLE I.—*Comparison of* B. pertussis *Antibody Response in Paired Sera by Complement Fixation (C.F.) and Agglutination Tests*

C.F. Titres	Agglutination Titres				Total
	Rising (⩾Fourfold)	High (⩾60)	Low (<60)	Neg. (<15)	
Rising (⩾fourfold)	*39*	8	5	5	57
High (⩾64)	5	*18*	6	0	29
Low (<64)	4	4	*26*	2	36
Neg. (<8)	0	1	4	*96*	101
Total	48	31	41	103	223

TABLE II.—B. pertussis *Isolations and Rising Titres*

B. pertussis Isolation	Rising Titres				Total
	C.F.+ Ag.+	C.F.+ Ag.−	C.F.− Ag.+	C.F.− Ag.−	
Positive	12	7	2	38	59
Negative	27	9	7	108	151
Total	39	16	9	146	210

TABLE III.—*Age of Patient in Relation to Isolation of* B. pertussis *and Serological Response*

Age	Total Patients		Patients with Positive Cultures		Patients with Positive Serology*		Patients with Positive Cultures and/or Serology	
	No.	%	No.	% in Age Group	No.	% in Age Group	No.	% in Age Group
<6 months	73	35	31	42	14 (2)	19	36	49
−1 year	26	12	7	27	7 (3)	27	12	46
−2 years	30	14	7	23	15 (7)	50	16	53
2-5 years	59	28	9	15	38 (11)	64	44	75
⩾5 years	22	10	5	23	19 (4)	86	19	86
Total	210		59		93 (27)		127	

*Rising or high (⩾60) titres by complement fixation and/or agglutination tests; numbers in parentheses indicate number with high titres.

TABLE IV.—*Previous Immunization in Relation to* B. pertussis *Isolation and/or Rise in Antibody Titre*

Previous Immunization	Culture Positive Rising Titre Positive	Culture Positive Rising Titre Negative	Rising Titre Positive Culture Negative	Total Patients
Full	6	1	18	25
Partial	2	0	2	4
Nil	10	35	17	62
	18	36	37	91

TABLE V.—*Previous Immunization and Age in* 91 *Children with* B. pertussis *Isolation and/or Rise in Antibody Titre*

Immunization	−6 months	−1 year	−2 years	2-5 years	⩾5 years	Total
Complete	2	3	2	9	9	25
Partial	2	0	1	1	0	4
Nil	30	8	7	15	2	62
	34	11	10	25	11	91

the diagnosis of whooping cough was confirmed by isolating *B. pertussis* the complement fixation test seemed rather more sensitive than the agglutination test in detecting rising antibody titres. Thus, when the complement fixation technique is convenient and practical, as in virus laboratories where paired sera from children with respiratory infections are tested against a battery of complement fixation antigens, a *B. pertussis* antigen could be useful.

We found that the age of the patient was of considerable importance in relation to both *B. pertussis* isolations and serological responses. Under 6 months of age isolation of *B. pertussis* was much commoner than positive serological findings; over 1 year of age serological tests were more often positive than were cultures. Our study suggests that two age-related factors were involved in this difference—namely, a poor or delayed antibody response by the young infant as has been previously reported in other respiratory infections (Grist, 1957; Chanock *et al.*, 1961; Ross *et al.*, 1964) and the low proportion of children under 6 months of age who had been fully immunized against *B. pertussis*. In some children previous immunization appeared to have suppressed growth of *B. pertussis* in the nasopharynx but not the serological response.

The preponderance of serotype 1,3 in both immunized and unimmunized patients is in keeping with previous findings reported by Preston (1963, 1965) and by the Public Health Laboratory Service Whooping Cough Committee and Working Party (1969). These reports indicated that the efficacy of much of the pertussis vaccine in use for some years before 1968 required improvement. Though since 1964 all manufacturers in the United Kingdom have included strains 1,3 in their *B. pertussis* vaccines (*British Medical Journal*, 1969) it would appear from the clinical and bacteriological findings of the present study that current whooping cough vaccines are still not adequately protective against strain 1,3.

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Outcome of Recent Thromboembolic Occlusions of Limb Arteries Treated with Streptokinase

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Summary: All our patients with a recent thromboembolic occlusion of limb arteries treated with streptokinase have been reviewed retrospectively. Clearing of the main artery, as judged by arteriography or reappearance of arterial pulsations, occurred more often when treatment was started early. If only patients with an iliac, femoral, or popliteal artery occlusion are considered, those who received a lower initial dose had a significantly higher clearing rate and a significantly lower mortality than those who received a high initial dose (500,000 units of streptokinase or more). Therefore an initial standard dose of 1,200,000 units of streptokinase is no longer recommended in these conditions, and even an individually titrated initial dose of more than half a million units could be hazardous. If no neurological abnormalities were present on admission amputation was never necessary, even if clearing of the main artery did not occur. If there was sensory loss of at least part of a limb, amputation was avoided only if the pulsations returned in at least one artery of hand or foot.

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Introduction

During the administration of streptokinase in patients with a recent thromboembolic occlusion of a limb artery, patency of the main artery can be restored (Fletcher *et al.*, 1959; Gross *et al.*, 1960; Kahn *et al.*, 1961; Cotton *et al.*, 1962; Nilsson and Olow, 1962; McNicol *et al.*, 1963; Salmon *et al.*, 1963; Schmutzler, 1963, 1968; Verstraete *et al.*, 1963; Winckelmann *et al.*, 1963; Marchal *et al.*, 1964; Haan and Tilsner, 1965; Hess, 1967). Nevertheless, neither the incidence of spontaneous disobstruction nor the optimal dose and duration of streptokinase therapy have yet been established. Furthermore, no controlled study is available that compares the value of streptokinase treatment with other current therapeutic regimens.

We have shown (Verstraete *et al.*, 1966), on the basis of arteriographic studies, that in these patients patency of the main artery can be obtained after administration of streptokinase according to the following standard dosage scheme: a set initial dose of 1,250,000 units and a maintenance dose of 100,000 units/hour for 72 hours. Since then more patients have been treated by this method. The aim of this paper is to describe the outcome in patients in whom recent thromboembolic occlusion of a limb artery was treated with streptokinase, and to compare the results obtained using