occurrence of this condition might explain some, though probably not all, examples of this phenomenon in the elderly. The mechanism of tuberculin insensitivity in sarcoidosis differs from the usual pattern in old age; thus Israel *et al.* (1950), Forgacs *et al.* (1957), and Scadding (1960) have all reported a common failure of tuberculin conversion after B.C.G. vaccination.

Declining Contact with Tuberculous Infection

Among those who showed tuberculin conversion after B.C.G. vaccination, and in whom the host response was therefore intact, it was most probable that this decline of tuberculin allergy with advancing age was the result of declining contact with *Mycobacterium tuberculosis*. This hypothesis fits well with the fact that tuberculin sensitivity in each age-group is weaker among women, who, for the most part, are less likely to be exposed to chance infection in the community. Moreover, as this phenomenon is observed well beyond the menopause it is difficult to explain this on an endocrine basis. In the minority who failed to show tuberculin conversion there must be some other factor. Our experience indicates that this is not in the skin, and we therefore assume a failure in the reticuloendothelial system to produce adequately sensitized lymphocytes.

Summary

Using the Heaf multipuncture test in 3,026 persons (1,469 aged 60 or over) we have confirmed declining sensitivity with advancing age, and this trend becomes discernible from the fifth decade onwards.

There was also a qualitative change in that those who were most sensitive (Grade 4 reactors) showed a progressive decline between the fourth and seventh decades.

When serial tuberculin tests in the *same* individual after a lapse of two years showed a change, tuberculin sensitivity was more likely to wane.

Those who were tuberculin-negative still showed normal immediate-type skin reactions when codeine phosphate and *Candida albicans* antigen were used.

Using special tuberculins, we failed to elicit any appreciable number showing latent tuberculin allergy. The delayed-type Koch phenomenon seen after B.C.G. vaccina-

tion is possibly a more critical test of latent tuberculin allergy.

There was a diminished capacity to achieve and maintain tuberculin conversion after B.C.G. vaccination in 124 persons, most of whom were elderly.

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TYPE-SPECIFIC IMMUNITY AGAINST WHOOPING-COUGH

BY

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Although whooping-cough in this country is less prevalent and usually less severe than it was before the introduction of pertussis vaccine, it still occurs, sometimes with fatal results, and moreover it occurs in children who have been vaccinated (Galbraith and Cockburn, 1963). One possible explanation of the occurrence of whooping-cough in vaccinated children is that the infecting bacterium may be different serologically from the organism with which the child has been immunized.

It was Andersen (1953) who first described the serological typing of freshly isolated strains of *Bordetella pertussis*, and her findings have been confirmed by Eldering *et al.* (1957), Preston and Te Punga (1959), and Lacey (1960). All strains possess a common antigen (antigen 1), but they may also possess one or more of a series of type-specific antigens (antigens 2, 3, and 4).

It has recently been shown (Preston and Evans, 1963) that these type-specific antigens play a part in immunity to experimental pertussis infection in mice, and the present communication presents evidence which suggests that they may also play a part in immunity to pertussis infection in children.

Materials and Methods

Strains.—Strains of *Bord. pertussis* were obtained from the Public Health Laboratories in Brighton, Bristol, Leeds, and Manchester; Hope Hospital, Salford; Royal Manchester Children's Hospital; Fazackerley Hospital, Liverpool; and from home cases in Manchester and Salford.

Typing Sera.—Antisera to four different serotypes of *Bord. pertussis* were produced in rabbits. These sera were freed from antibody to heat-stable antigens by absorption with heated suspensions of the homologous strains. They

were then further absorbed with unheated suspensions of heterologous strains so as to produce monospecific sera, each containing antibody to one of the heat-labile antigens (1, 2, 3, or 4). The technique was the same as that described by Preston and Te Punga (1959), except that autoclaved suspensions were used instead of steamed suspensions for the removal of antibody to heat-stable antigens, since it was found that steaming did not always completely destroy the heat-labile antigens.

Each monospecific serum was finally diluted so that it had an agglutinin-titre of 320 when tested against a strain of *Bord. pertussis* which had the homologous antigen as a major antigen.

Serological Typing by Slide-agglutination.—A suspension of each strain of *Bord. pertussis*, grown on charcoal-blood-agar, and each vaccine (standardized to contain approximately 50,000 million organisms per ml.) was mixed with a standard loopful of each of the four monospecific sera on a glass slide and rocked gently for five minutes. Degrees of agglutination were recorded as follows:

- +++ , Rapid formation of large agglutinates, complete within 3 minutes.
- ++ , Agglutinates visible within 3 minutes; complete within 5 minutes.
- + , Slow formation of small agglutinates, incomplete within 5 minutes.
- 0 , No agglutination visible to naked eye within 5 minutes.

All the tests recorded in the tables were performed within two days, using the same four batches of absorbed monospecific sera.

Results

Typing of Strains

The results of typing strains isolated in Manchester before 1958 and those isolated in Leeds in 1960 are given in Table I. It is clear that in general the strains isolated in Manchester had a different antigenic pattern, especially with regard to antigens 2, 3, and 4, from those isolated in Leeds.

TABLE I.—*Antigenic Composition of Pertussis Strains Isolated Before 1958 and in 1960*

Strain of Pertussis	Isolation		Agglutination by Antibody			
	Place	Year	1	2	3	4
BH	Manchester	Before 1948	+++	+++	+++	+++
FF	"	"	+++	+++	0	+++
ML	"	"	+++	+++	0	++
PB	"	"	+++	+++	0	++
PE	"	"	+++	+++	0	+++
SY	"	"	+++	0	++	0
SN	"	"1957"	+++	+++	0	+++
14	Leeds	1960	+++	++	+++	+
26	"	"	++	0	++	0
43	"	"	+	0	++	0
50	"	"	++	0	++	0
53	"	"	+	0	+++	0
61	"	"	+++	0	++	0
72	"	"	+++	0	+++	0
73	"	"	+	0	++	0
79	"	"	++	0	++	0
85	"	"	+	0	++	0
87	"	"	+	0	++	0

For significance of degrees of agglutination, see text.

Andersen (1953) demonstrated that each local outbreak of whooping-cough is caused by a single serotype of *Bord. pertussis*, and it may have been that the serotype which caused the cases in Leeds in 1960 was uncommon in Manchester in earlier years. There was also the possibility, however, that the prevalent serotype throughout the country had changed as a result of the use of pertussis vaccines.

It was therefore important to examine strains from various parts of the country and to note any differences

between those isolated from vaccinated and unvaccinated persons. Fourteen strains from six different localities were examined (Table II) and all, as with those isolated in Leeds in 1960, had the common antigen 1 and antigen 3 but only five had antigens 2 and 4. None of the three strains from patients who had been immunized with pertussis vaccine had antigen 2 or 4.

TABLE II.—*Antigenic Composition of Pertussis Strains Isolated Since 1960*

Strain of Pertussis	Isolation		Patient Immunized with Pertussis Vaccine	Agglutination by Antibody			
	Place	Date		1	2	3	4
JG	Manchester	Jan. 1961	No	+++	+++	+++	++
TO	"	Feb. 1963	"	+++	+++	+++	+++
GB	Salford	Jan. 1963	Yes	+	0	+++	0
DB	"	Mar. 1963	"	++	0	++	0
PT	Brighton	"	No	+++	0	+++	0
DS	Bristol	Jan. 1963	Yes	++	0	+++	0
LS	Leeds	Feb. 1963	No	+	0	+++	0
NM	Liverpool	"	"	+++	+++	+++	+++
TD	"	"	"	+++	+++	+++	+++
MB	"	"	"	++	0	+++	0
MW	"	Apr. 1963	"	+++	+++	+++	+++
JT	"	"	"	++	0	+++	0
RP	"	"	"	+++	0	+++	0
SS	"	"	"	++	0	+++	0

For significance of degrees of agglutination, see text.

Antigenic Content of Vaccines

The antigenic content of the vaccines with which the three patients GB, DB, and DS (Table II) had been immunized was not known, but tests were made on seven batches of vaccine from four British manufacturers (A, B, C, and D). The results are shown in Table III.

TABLE III.—*Antigenic Composition of Pertussis Vaccines*

Vaccine	Manufacturer	Batch	Year of Manufacture	Agglutination by Antibody			
				1	2	3	4
British	A	1	1949	+	++	0	+
"	A	2	1963	+++	+++	0	++
"	B	1	1955	++	+++	0	++
"	C	1	1955	+++	+++	++	+++
"	C	2	1955	+++	+++	++	+++
"	D	1	1955	+++	+++	0	+++
"	D	2	?1955	++	++	0	++
American			1956	++	++	++	0
French			1956	+++	+	0	0

For significance of degrees of agglutination, see text.

Only the two batches produced by manufacturer C contained antigen 3, while the other five batches, which included one in current use in this country, were deficient in this antigen. Similar tests were made on one American and one French vaccine, both of which proved to be deficient in antigen 4; the French vaccine was also deficient in antigen 3.

TABLE IV.—*Antigenic Composition of Pertussis Strains Used by Manufacturers of Vaccine in 1955*

Manufacturer	Strain of Pertussis	Agglutination by Antibody			
		1	2	3	4
B	CN	+++	+++	0	+
B	HS	+++	+++	0	++
B	LN	+++	+++	0	++
B	ON	+++	+++	0	+++
B	PN	+++	+++	+++	+
B	SE	+++	+++	+++	+
C	12	+++	+++	+++	+++
C	13	+++	+++	0	+++
C	81	+++	+++	0	+++
C	83	+++	+++	0	+++
C	91	+++	+++	0	+++
C	70	+++	+++	+++	+
C	71	+++	+++	0	+++
C	74	+++	+++	0	+++
C	75	++	0	++	0
C	92	+++	+++	+++	+++

For significance of degrees of agglutination, see text.

Our departmental collection of pertussis strains included ten which were used by manufacturer C to produce vaccines in 1955, and six which were used by manufacturer B. These were likewise subjected to slide-agglutination, with the results given in Table IV.

Clearly, an individual batch of vaccine may not necessarily contain the full range of type-specific antigens.

Discussion

The results of this investigation suggest that the strains of *Bord. pertussis* frequently encountered in this country in the past, and probably used in the manufacture of pertussis vaccines, contained predominantly antigens 1, 2, and 4 and less frequently antigens 1, 2, 3, and 4. Vaccines deficient in antigen 3 may give incomplete immunity against infection by strains containing antigens 1 and 3, which appear to be causing most cases of whooping-cough in this country at the present time. Attention is drawn particularly to the three strains (all type 1, 3) isolated from vaccinated patients, one of whom had a severe infection.

The weakness of the argument lies in the relatively small number of strains and vaccines which have been examined. However, the object of this communication is to indicate the need for a fuller and more widespread investigation of freshly isolated strains of *Bord. pertussis* and comparison of their antigenic composition with that of pertussis vaccines, not only in this country but also in other countries where vaccination is less effective than may be expected (Petrilla and Barsy, 1959; Edsall *et al.*, 1962).

This investigation has been confined to the heat-labile antigens originally designated by Andersen as (major) antigens 1, 2, 3, and 4; it has not included other heat-labile (minor) antigens which are capable of producing only low titres of antibody (Andersen, 1953; Eldering *et al.*, 1957; Preston and Te Punga, 1959). Even antigen 4 has been considered a minor antigen by Eldering *et al.* (1962), and with this I would agree, for we have never produced high titres of antibody 4 in our rabbits. Moreover, no strains have been found to possess antigen 4 in the absence of antigen 2. It seems, then, that, in this country at least, investigations could be confined to the major antigens (1, 2, and 3) unless freshly isolated strains are shown to produce high titres of some other antibody.

One of the difficulties in acquiring recently isolated strains of *Bord. pertussis* at the present time is that some laboratories, having isolated an organism from a case of whooping-cough, have been uncertain about its identity. Although a strain may appear typical both morphologically and culturally, it may fail to agglutinate with standard anti-pertussis serum. It is possible, as suggested by Lacey (1957), that the anti-pertussis serum may have been produced against a strain containing antigens 1, 2, and 4, but no 3; such a serum would contain no antibody 3 and, as shown by Andersen (1958), it may also have very little antibody 1. It could not, therefore, be expected to give good agglutination of strains such as GB or LS (Table II) which are strongly agglutinated only by antibody 3. This emphasizes the necessity for diagnostic pertussis serum to contain an adequate amount of antibody 3.

Summary

A number of pertussis vaccines and strains of *Bordetella pertussis* were examined for their content of heat-labile antigens 1, 2, 3, and 4, by agglutination with specific sera.

Differences in antigenic composition were found between strains encountered in the past and those being isolated at the present time.

It is suggested that antigen deficiencies in pertussis vaccine may result in failure to give complete protection against whooping-cough if the infecting strains of *Bord. pertussis* possess antigens not present in the vaccine.

This investigation would not have been possible without the ready co-operation of medical practitioners and technicians whose help and assistance are gratefully acknowledged—namely, Sir Graham Wilson and the staff of the Public Health Laboratory Service; Salford Health Department and many general practitioners in Manchester and Salford; the staff of Fazackerley Hospital, Liverpool; the laboratory staff of the Royal Manchester Children's Hospital and of Hope Hospital, Salford; and Mrs. Pamela Garrity and Miss Joyce Crompton of this department.

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IDIOPATHIC RHABDOMYOLYSIS (MYOGLOBINURIA) AS AN ACUTE RESPIRATORY PROBLEM

BY

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A rare cause of peripheral respiratory failure is idiopathic rhabdomyolysis. This unusual disease (reviewed by Korein, Coddon, and Mowrey, 1959) is due to dissolution of striated muscle and excretion of the muscle protein, myoglobin, in the urine. Hence this syndrome, long referred to as "idiopathic paroxysmal myoglobinuria," has been renamed rhabdomyolysis (Bowden, Fraser, Jackson, and Walker, 1956). The disease is intermittent in character, the exciting factor often being physical exertion or, less often, a febrile illness. The large muscles of the extremities, usually the lower, bear the brunt of the disease. Pain and swelling in the affected muscle group are followed by the excretion of characteristically dark-red urine (myoglobinuria) which soon turns brownish-black on standing. In only very rare instances are the muscles of respiration involved so that respiratory failure dominates the clinical picture. Such a case, simulating bulbospinal poliomyelitis, is presented, together with details of its management.

Case Report

A 22-year-old married woman was admitted to hospital complaining of difficulty with breathing, talking, and swallowing