THE IRISH JOURNAL OF MEDICAL SCIENCE
THE OFFICIAL JOURNAL OF THE ROYAL ACADEMY OF MEDICINE IN IRELAND.

SIXTH SERIES. No. 316. APRIL, 1952.

RECENT ADVANCES IN PERTUSSIS IMMUNISATION.*

By J. UNGAR.

STATISTICS from several countries show that pertussis is among the most debilitating of diseases in children, being a frequent cause of death in the first year of life. One of the obvious ways to combat the disease is by preventive inoculation of children at an early age.

Since Nicolle and Conor reported their first results in immunising against pertussis in 1912, and the reports, largely confirmatory, by Madsen in 1923-24 of the benefits of vaccination to the child population of the Faroe Isles, an extensive literature has arisen on the problem. Many publications in the early 1930's showed inadequate epidemiological follow-up of the tests; others lacked evidence of the suitability of the antigens used. Thus the use of prophylactic inoculation against pertussis came into some disrepute. When the existence of different phases of *H. pertussis* was established (Leslie and Gardner, 1931), however, an important criterion became available in the selection of strains suitable for the production of efficient antigens, and reports published during the last decade give ample evidence that real protection has been achieved in many large-scale trials (Kendrick, Sauer, Foley, Holt, Medical Research Council). Most of the recent publications indicate that the incidence of pertussis after treatment with an effective vaccine is lowered in a large proportion of the inoculated children and also that the severity of the disease is lessened in those inoculated children who do contract the disease.

There is now convincing evidence to show that *Hæmophilus pertussis* is the sole cause of whooping-cough and that no virus is involved. Thus volunteers have been experimentally infected with *H. pertussis* suspensions grown on artificial media: both patients and convalescents have in their blood-streams specific antibodies against *H. pertussis*; in 90 per cent. of cases the bacterium itself can be isolated by the swab and cough-plate method.

At the outset I would emphasise that laboratory tests, whether in the test-tube or on experimental animals and however elaborate, can give only a general indication of a vaccine's protective value. The ultimate criteria of its efficacy are the incidence and severity of the disease in inoculated and uninoculated children after exposure to infection.

Various products, such as vaccines, toxoids and mixed vaccines and

* From Glaxo Laboratories Ltd., Greenford, Middlesex, England. Communication made to the Section of Public Health, Royal Academy of Medicine in Ireland, December 17th, 1951.

toxoids, are available for immunisation, and it is sometimes difficult to decide which to use. Fortunately knowledge acquired during the last few years of the biology and antigenic structure of *H. pertussis* has greatly contributed to the development of vaccines and to showing the best means of testing the efficacy of different antigens in the laboratory. It seems that, despite the many attempts to prepare antigenic fractions from *H. pertussis*, the intact bacterial cell is still the best; in our experience fractions (endotoxoid, agglutinogen) were inferior immunising agents in laboratory animals.

The following is an account of investigations into some of the factors that we consider important for the production of effective antigens.

(1) *Choice of strains.*

It is necessary to use smooth strains showing typical growth and morphology. Ungar and Stevens (1951) examined over 200 *H. pertussis* cultures and confirmed (Kristensen, 1922, 1927) that freshly isolated strains form a single antigenic type, which differs from the avirulent and *H. parapertussis* strains. *H. pertussis* strains are agglutinated to a high titre (up to 1 : 100,000) with immune serum, are virulent to mice by intranasal or intracerebral inoculations and produce dermonecrotic lesions in the rabbit's skin. Virulent, but not avirulent, *H. pertussis* strains agglutinate red cells (hæmagglutination) and grow on solid media only in the presence of blood or in fluid media after the addition of starch (Cohen and Wheeler, 1946). Further differentiating features are the precipitation of suspensions of virulent strains by a 2 per cent. suspension of aluminium phosphate and the solubility of virulent strains in dilute sodium hydroxide solutions (Ungar and Muggleton, 1949).

Under laboratory conditions, avirulent variants of virulent strains can be produced, e.g., by growing virulent strains on unfavourable media, and they conform with the description of phases III or IV given by Leslie and Gardner (1931). This change is irreversible and the variants are antigenically distinct from the virulent strains. In immunological investigations the two types, virulent and avirulent, form two separate and easily distinguishable entities. There is no real proof that phases of *H. pertussis* occur naturally and we have to assume that these were artificially produced avirulent variants, whereas freshly isolated strains form a single antigenic type with characteristic physical and immunological properties. These strains can be freeze-dried, when they maintain their biological properties unaltered over a long period (for at least two years and often much more). Great merit attaches to Leslie and Gardner's (1931) demonstration that there exist atypical forms of *H. pertussis*, produced under laboratory conditions and antigenically distinct from virulent *H. pertussis*.

The selection and maintenance of suitable strains are essential for producing effective vaccines. The degree of agglutinability of strains and their virulence to mice, as determined by the intranasal method of Burnett and Timmins (1937) or the intracerebral method of Kendrick *et al.* (1947), are often closely related, but there are strains of low agglutinability (1 : 3,000) having high virulence to mice and good antigenic properties.

(2) *Suitable media for maintaining antigenicity.*

Bordet-Gengou medium with the addition of human or animal blood is still generally used for vaccine production, although there are indications that liquid media (Hornibrook, 1939; Cohen and Wheeler, 1946; Ungar *et al.,* 1950) may be more widely used for their obvious advantage of dispensing with the need for blood as an essential component of the solid medium. Comparative tests have shown that cultures grown for 24-48 hours on solid media at 35-37°C. have the same antigenicity in experimental animals as those grown in static fluid cultures for five or six days.

(3) *Harvesting of suspensions and killing methods.*

Sauer, who used the unwashed suspension for vaccine production, stated (1934) that the supernatant fluid of the unwashed suspension contained an appreciable amount of soluble toxin. This was later confirmed (Miller and Faber, 1939; Ungar and Jenner, 1943), and it was shown that the supernatant fluid is also highly antigenic. It is, therefore, advisable to wash the suspension as little as possible and to use saline rather than distilled water.

Apart from mechanical disintegration (used for the production of endotoxin or soluble fractions), such as grinding the bacteria, ultrasonic disruption or freezing and thawing, any common antiseptic (phenol, formalin, mercurial compounds) or heat may be used to sterilise the suspensions. We have investigated the effect of some antiseptics on the antigens in the bacterial cell and have found that phenol seems to affect the agglutinogen of the *H. pertussis* more than does merthiolate or formalin.

TABLE I.

24 hours' growth *H. pertussis* suspensions killed by . . .	Agglutination titre
0·2% formaldehyde	1 : 25,000
0·5% phenol	1 : 400
0·01% merthiolate	1 : 12,500

When testing vaccines for their antigenic properties in laboratory animals, we have found that vaccines killed with merthiolate and formol gave the same degree of protection, the next most effective methods being those with phenol, alcohol (75 per cent.) or heat. But whereas vaccines killed by heat (60°C. for 30 minutes), phenol, formalin or alcohol are sterile and, after 24 hours' standing at room temperature, non-toxic to mice or guinea pigs, the merthiolate-treated vaccine is still viable and toxic, the toxicity disappearing gradually over four weeks. The toxicity to mice of the vaccines freshly treated with merthiolate is due to the "endotoxin ", which is quickly inactivated by heat, phenol and formalin, but only slowly by merthiolate.

The existence of a toxic fraction in *H. pertussis* had already been demonstrated by Bordet and Gengou (1906) and later studied in detail by a number of investigators (Evans and Maitland, 1937; Ehrich *et. al.,* 1942), who gave methods of liberating the toxic fractions and described

their properties. The pathogenic properties are best demonstrated by the dermonecrotic reaction in the rabbit's skin and by the effect on mice after systemic injection. Our experience indicates that the toxic action of *H. pertussis* is caused by a water-soluble fraction possessing many characteristics of an endotoxin. The toxic fraction is prepared by grinding a dried suspension of *H. pertussis* in an agate ball-mill; about 1 mg. (expressed as freeze-dried powder) is thereby extracted from $25{,}000 \times 10^6$ organisms.

The dried powder preserves its toxicity for at least one year; in aqueous solutions its activity drops after seven days by more than 99 per cent. The LD 50 to mice after intravenous injection is about 0·65 mg of the toxin per kg. body weight. A dermonecrotic reaction on rabbit skin can be produced by an intracutaneous injection of 0·1 ml. of a diluted solution of the toxin (up to 1 : 5,000). These two properties of the fraction can be produced by injection of an equivalent amount of a live culture or of a vaccine freshly killed with merthiolate. The toxic fraction is heat labile; warming for 30 minutes at 45°C. detoxifies it. We have found that the toxic fraction is not filtrable, for a filtrate through Ford's Sterimat filters (3·6 cm.) had lost its toxic action, and the same is true of filtration through a $2'' - \frac{1}{2}''$ Berkefeld diatomaceous earth type of filter or through a Chamberlain porcelain filter. The toxic fraction does not contain histamine-like substances (test on cats) and is not pyrogenic (test on rabbits). It is detoxified on addition of 0·2 per cent. formaldehyde, particularly fast when kept at 37°C., although some batches of the toxic fractions have required up to ten days' incubation for complete detoxification. After partial detoxification the hæmorrhagic reaction disappears, but the necrotising factor disappears only when the toxic fraction has become completely inactive.

The dermonecrotic and lethal effects of the toxic fraction are neutralised by the addition of specific immune serum (antibacterial, anti-endotoxic), but no flocculation occurs in the tubes when the immune serum is added to serial dilutions of the toxic fraction.

It seems from our experiment on the nature of the toxic fraction that there is no readily secreted toxin in the bacterial cell. The *in vitro* tests and the responses of immunised animals indicate that there is no experimental basis for assuming any antitoxic immunity to pertussis. Antigens prepared from disrupted bacteria, which contain the toxic fraction, give a varying degree of "antitoxic" response in animals, but the immunity induced by these fractions in animals is predominantly antibacterial (agglutinins, protective antibodies). We have confirmed the earlier results of Evans (1940) and others (Ehrich *et al.*, 1942), who showed that the fractions might be used as immunising agents, but that the resulting immunity is antibacterial and not merely antitoxic.

Presentation of the Vaccine.

Vaccines may be used either as plain suspensions or adsorbed on a mineral carrier. We have been experimenting for some years with different precipitating agents under varying conditions (e.g., potassium aluminium sulphate, ammonium sulphate, zinc sulphate, ethanol) and we finally chose an aluminium phosphate precipitated vaccine, as this gave an optimal response in animals. The mode of preparing this

vaccine differs from that of Harrison *et al.* (1938). The reason for adopting aluminium phosphate precipitation was not only that precipitated antigens are slowly absorbed from the depôt of injection, thus prolonging the antigenic stimulus, but also that it made possible combination of the vaccine with precipitated diphtheria toxoid. Recent reports (Sako, 1947; Sauer, 1946) indicate that babies of two to three months give a better immunity response after injection of the aluminium phosphate precipitated vaccine than after a plain suspension. It seems reasonable to assume, therefore, that the plain *H. pertussis* suspension will be used for single inoculations, but that the precipitated vaccine will be used for the inoculation of very young babies or as an addition to precipitated diphtheria toxoid for combined immunisation.

Methods of Testing Antigenic Activity.

Another factor making for further progress in the field of pertussis immunisation is the experience gained with methods now available, the tests on mice and rabbits for differentiating between active and less active products. Guineapigs, rats and hamsters have been found less suitable for the purpose. The most important tests are those carried out on mice, which can be used not only to establish the virulence of *H. pertussis* strains, but also for comparative evaluation of the protective power of vaccines. It has taken a long time to develop an animal test for vaccine standardisation, in marked contrast to what happened with diphtheria antigen, for which the guineapig test has been successfully used for many years. We have found that different strains of mice differ in their susceptibility to *H. pertussis* infection and also in their immunity response to the injection of *H. pertussis* antigens. The experiment cited below shows that of the four strains of mice tested, the "Swiss" was suitable for our purpose. Groups of twelve anæsthetised mice of the same age were infected intranasally with a fresh suspension of *H. pertussis* and survival rates were recorded.

TABLE II.

Strain of Mice	Infecting Dose × 10^6	No. of Mice	
		At start	Surviving 7 days
Agouti (Strong CBA) ..	200	12	12
	20	12	11
Black (Little C57)	200	12	12
	20	12	12
White (Strong A2)	200	12	7
	20	12	9
White—"Swiss"	200	12	4
	20	12	6

The description in brackets is of the original strain from which the stock has been maintained by continuous brother-sister mating.

On the basis of this experiment we adopted the susceptible "Swiss" mice as test animals. It should also be emphasised that the type of diet on which mice are kept may influence their susceptibility to infection

with *H. pertussis,* and it is advisable to have this factor standardised before any investigation is begun and so avoid disturbing effects due to changes in diet during the experiments.

Most workers use mice extensively both for virulence determination and for antigenicity studies by the intranasal (Burnet and Timmins, 1937), intracerebral (Kendrick *et al.,* 1947), intratracheal (Bradford, 1938) or intraperitoneal route (with the addition of mucin (Miller, 1933)) for challenging immunised mice with a live culture. We have chosen the intranasal route for assaying the virulence of *H. pertussis* strains and the intracerebral route for challenging immunised mice to determine the degree of protection conferred on them. Table III gives the result of one such test. Groups of 15 mice were injected intraperitoneally with a single dose of graded amounts of a vaccine in 0·2 ml. of saline; ten days later the animals were challenged intracerebrally with at least one hundred times the LD 50 of a virulent strain. Survival rate fourteen days after the challenge was recorded.

TABLE III.

PROTECTION OF MICE

from *H. pertussis* by intraperitoneal injection of vaccine
(15 animals per dose).

Controls : 500 organisms in 0·05 ml. (intracerebrally)—66% survival.
5,000 ,, ,, ,, ,, —13% . ,,
Challenge dose : 50,000 organisms in 0·05 ml.

Vaccine	Immunising Dose	Survival on 14th day
	$\times 10^6$	(per cent.)
A III	40	13
	200	46
	1,000	60
B III	40	13
	200	60
	1,000	93
C III	40	20
	200	53
	1,000	100
E IV	40	0
	200	46
	1,000	73
B IV	40	33
	200	60
	1,000	93

It is clear from the table that the vaccines used gave a well-graded response.

The rabbit, in our experience, is a suitable test animal for measuring the antigenic efficiency of both the antibacterial and anti-endotoxic components of vaccines. We have found that the combined tests on mice and rabbits enable us to obtain a fairly precise evaluation of the activi-

ties of the different antigenic fractions. Groups of at least three rabbits are injected intramuscularly with four doses ($5{,}000 \times 10^6$) of the antigen at weekly intervals; ten days after the last dose blood is withdrawn from the rabbit and the serum is separated and tested for (*a*) agglutinin content (the adequate titre is regarded as 1 : 5,000); (*b*) complement fixing antibodies (1 : 250); (*c*) protective antibodies. Groups of twelve mice are injected intraperitoneally with 0·1 ml. of the immune serum and an hour later they are infected intranasally or intracerebrally with a fresh suspension of *H. pertussis*. The rate of survival depends on the potency of the serum. We have observed that the protective properties of the serum usually correlate well with the levels of the agglutinins. Table IV gives an example of the tests in which inoculated mice were challenged intracerebrally.

TABLE IV.

Sera from rabbits immunised with . .	Percentage of mice surviving 14 days after challenge.
Endotoxin from virulent strains	70
,, ,, avirulent ,, ...	10
Vaccine from virulent strain	100
,, ,, avirulent ,, ...	0
H. parapertussis vaccine	0
Normal rabbit serum	0
Control : 50,000 organisma without serum	0

In addition, the endotoxin neutralising effect of the sera is estimated by using the skin in rabbits and the intraperitoneal route in mice. The endotoxin (giving, in dilutions up to 1 : 5,000 in 0·1 ml., a positive skin test in rabbits and killing mice after intraperitoneal injection) is added to a serial dilution of the immune serum in Lambert tubes, which are then well shaken and incubated for one hour at 37°C. The mixture is injected in quantities of 0·2 ml. into the depilated flank of a rabbit and intraperitoneally into groups of six mice.

Table V gives the results of one such experiment.

TABLE V.

Immune Serum	Reaction in Rabbit Skin	No. of Mice	Mice surviving 7 days after injection
Dil. 1/1	— — — —	6	6
1/5	— — — —	6	6
1/10	— — — —	6	6
1/20	— — — —	6	3
Normal Serum ..	+ + +	6	0
Saline Control ..	+ + +	6	0

We have found that the agglutination titre of different rabbit immune sera is correlated with the potency of the endotoxin antibodies in the

same serum. Table VI shows the endotoxin neutralising properties of the rabbit sera and the corresponding agglutination titres.

TABLE VI.

	Dilution of serum neutralising the endotoxin				Agglutination titre
	1/2	1/4	1/10	1/20	
Control serum	++	++	++	++	Less than 1/100
Immune serum Batch 8	0	0	+	++	1 : 1,600
,, ,, ,, 6	0	0	0	±	1 : 6,400
Purified immune serum	0	0	0	0	1 : 12,800

Rabbit immune sera differ from human immune sera (convalescent or hyperimmune), in which hardly any "antitoxin" can be demonstrated, although agglutinins, complement fixing antibodies and antibacterial antibodies are all present (Evans, 1940; Ehrich *et al.*, 1942; Ungar and Jenner, 1943). In our tests with sera from children convalescent from whooping cough and with concentrated human globulins (Eli Lilly or Armour Laboratories) we have shown the presence of some agglutinins and protective antibodies but no antibodies neutralising "endotoxin". It may be mentioned that Demnitz and Becker (1939) have reported that horse immune serum containing pertussis antitoxin is effective in mice but not in humans. It seems that there is no proof yet that antitoxic immunity in humans is of basic importance.

In testing the combined pertussis-diphtheria antigen, guineapigs are used to determine the potency of the toxoid. Antitoxin titration in the serum of immunised guineapigs (under the T.S.A. Regulations) has shown not only that the two antigens are compatible (Schütze, 1940), but also that the addition of the vaccine enhances the antitoxic response. The addition of any bacterial suspension (*Escherichia coli, Serratia marcescens*) acts in the same way as the addition of a mineral carrier. Table VII clearly demonstrates the effect and also that the amount of the carrier (Bousfield and Holt, 1947) and possibly the particle size of the absorbing agent influence the level of antitoxin.

TABLE VII.

Lf per ml.	Final conc. of organisms $\times 10^6$	Final conc. $AlPO_4$ mg/ml.	No. of guinea pigs	Units diphth. antitoxin per ml. geom. mean.
50	Nil	Nil	10	< 0·75
50	,, ...	5	10	1·38
50	,, ...	10	10	2·26
25	*H. pertussis* suspension 20,000	5	10	6·07
25	*E. coli* ,, ,,	5	10	2·16
25	*Ser. marcescens* ,, ,,	5	10	3·46
25	Small collodion particles 20,000	5	10	2·20
25	Large ,, ,, ,,	5	10	2·17
25	Yeast suspension 20,000 ..	5	10	8·50

Greenberg and Fleming (1947) have drawn attention to the fact that

graded doses of *H. pertussis* suspensions increase the immunising effect of the fluid toxoid, and we have confirmed this.

TABLE VIII.

Dipth. toxoid Lf ml.	$AlPO_4$ conc. mg/ml.	*H. pertussis* vaccine $\times 10^6$	No. of guinea pigs	Dipth. antitoxin units/ml. of serum geom. mean.
50	0	0	10	1·00
25	2·5	0	10	1·85
25	5·0	0	10	2·41
25	10	0	10	2·9
25	0	20,000	10	2·87
25	2·5	20,000	10	2·34
25	5·0	20,000	10	2·00
25	6·6	20,000	10	2·41

It seems (Table VIII) that in the combined diphtheria-pertussis vaccine the amount of aluminium phosphate can be reduced or it can be omitted, without causing any drop in antigenic efficiency of the diphtheria toxoid. Tests on rabbits and guineapigs injected subcutaneously with the combined antigen and varying amounts of the mineral carrier have shown that the incidence of nodule formation decreases with the reduction of the aluminium phosphate content in the combined prophylactic. Trials on children will be needed to show whether or not the combined immunisation with pertussis-diphtheria antigens with reduced amounts of the mineral carrier, or without carrier, can give as good protection against diphtheria as the precipitated toxoids.

Attempts to determine the suitability of skin tests for assessing an acquired immunity in rabbits gave results too conflicting to be of practical value. We tried the toxin test suggested by Strean (1940), bacterial suspension and the agglutinogen test (Flosdorf *et al.*, 1943), but we could not reproduce the specificity of the skin test described by these authors. It seems, therefore, that the response of humans to the suggested skin tests is based on their more specific response (probably allergic reactions) to the bacterial cell than occurs in the rabbit; further field trials may provide convincing evidence about the diagnostic value of the skin test in pertussis.

Factors Influencing the Degree of Immunity.

The results of animal experiments may be conveniently applied to the clinical aspects of active immunisation against pertussis. We have seen in Table III the graded responses of mice to increases in the immunising dose, and experience has shown that the size of the actual immunising dose has an important bearing on the induction of an adequate response in vaccinated children.

The total quantity of the plain suspension recommended for immunisation is about $100{,}000 \times 10^6$ organisms, divided into three or four doses. Kendrick recommends a total of $60{,}000 \times 10^6$ (1936), and the results of the M.R.C. trial (1951) show conclusively that this is adequate. Experiments on mice have shown that a single dose of an aluminium phosphate adsorbed vaccine will give the same protection as three to four times

the dose of the plain suspension, and this again may explain why, in children, the total dose of the aluminium phosphate adsorbed vaccine can be reduced to $30{,}000 \times 10^6$ organisms (Bell, 1942) and still give the same final response. There is no evidence available to permit a decision whether or not the size of the doses of vaccine for the primary and secondary stimulus is as important as in immunisation with diphtheria toxoid (Barr *et al.*, 1950).

The correct spacing of doses undoubtedly has a bearing on the success of immunisation, particularly when an aluminium phosphate precipitated vaccine or a combined prophylactic is used, and the recommended interval between doses is four weeks or more. Animal experiments well illustrate the significance of this time interval (Table IX).

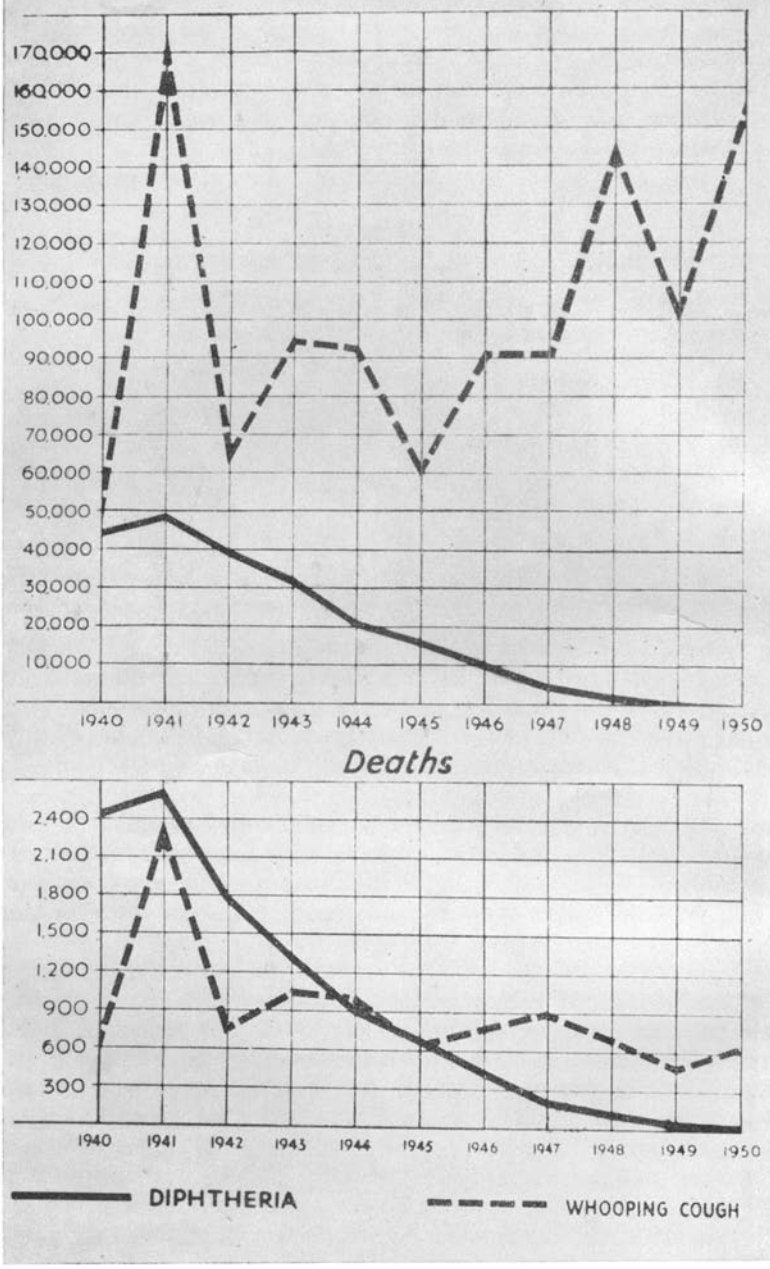
TABLE IX.

Vaccine	Immunisation route	Dose in 10^6	No. of mice	Interval between immunisation and challenge (weeks)	No. of mice surviving after 14 days
Plain suspension ..	i.p.	1,000	10	2	9
,, ,, ..	,,	,,	,,	4	8
Alum. phosphate precipated	,,	,,	,,	2	2
Alum. phosphate precipated	,,	,,	,,	4	7
Control (saline) ..	,,	—	,,	2	0

Not only did simultaneous immunisation against whooping cough and diphtheria prove that they are compatible, but there is also ample laboratory evidence (Tables VII and VIII) and there are clinical reports (di Sant' Agnese, 1949) to show that the addition of the pertussis vaccine considerably increases the antitoxin response to the diphtheria toxoid.

It is now recognised that the proper age for immunisation against pertussis is the first year of life, preferably at two to four months old, since it takes about one to two months after completion of the treatment for immunity to become well established, depending on the type of vaccine used. Undoubtedly, the choice of antigen will depend on the age of the child. When only pertussis immunisation is intended, the aluminium phosphate precipitated vaccine seems to be preferable to plain suspensions for infants of the age of two or three months (Sako *et al.*, 1945). It is still a question whether combined pertussis-diphtheria immunisation should be performed in these very young infants, in view of reports (Barr *et al.*, 1950; Vahlquist, 1949) of the presence of diphtheria antitoxin in naturally immune babies up to six months of age; only clinical trials with suitable antigens can provide the answer. There is one other factor bearing on the age of immunisation and that is the danger of poliomyelitis infections. The incidence of poliomyelitis in young babies is lowest, whereas pertussis mortality is highest. It would seem, therefore, that combined immunisation could be performed with no fear of poliomyelitis complications in babies of six months, and

DIPHTHERIA AND WHOOPING COUGH CASES NOTIFIED IN ENGLAND AND WALES



preferably under, should clinical trials prove satisfactory. If this is done, a booster dose would be advisable about twelve months after completion of the immunisation.

General reactions (malaise, pyrexia) after injection of vaccines have seldom been encountered, but occasionally after injection of the aluminium phosphate adsorbed antigen there have been local reactions in the form of nodules (foreign body reactions) or rarely as sterile abscesses. These reactions can be cut down with a proper technique of injection (preferably intramuscular); the container should be well shaken to distribute the antigen evenly and the right kind of needle must be chosen.

Laboratory tests of acquired immunity to pertussis were suggested and are still in use, such as serological tests for agglutinin titration, complement fixation and opsono-phagocytic index, but their practical application is limited, as they require the taking of blood samples from babies. The diagnostic value of the skin test with the agglutinogen (Flosdorf *et al.*, 1943) needs further verification.

Serum treatment in pertussis has a certain value in the prophylaxis of contact cases in the first six or seven days of the incubation. The protective action, which is mainly antibacterial, can be shown experimentally in infected animals (see Table IV) to be limited to the first few days of infection. Reports on serum treatment of severe pertussis are, however, mostly unfavourable.

The value of large-scale inoculation has been estimated by comparing the communicability rate in the treated and control groups and their respective morbidity and mortality rates (Crawitz and Cauley, 1945; Foley, 1946; Kendrick and Eldering, 1936; M.R.C., 1951).

The recently published report by the M.R.C. (1951) shows that the vaccines used produced satisfactory protection of the inoculated children. The reduction of the incidence of the disease was highly significant in the treated groups (78 per cent.), for the attack rate in the vaccinated children was 1·45, compared with 6·72 in the unvaccinated. The attack rate in vaccinated children exposed to pertussis in their homes was 18·2 per cent., against 87·3 per cent. in the unvaccinated group. The course of the disease in the vaccinated children who happened to contract it was considerably milder than in the unvaccinated. It appeared also in the M.R.C. trials that there was no evidence of any waning in degree of protection during the period of observation (2-3 years).

The notification records of pertussis show that, although the mortality from the disease has been steadily declining, its incidence fluctuates considerably and was still high in 1950 (see figure attached). One of the obvious methods of combating this disease, even considering all the prospects of effective chemotherapy, is prophylaxis to the same extent as against diphtheria, by the large-scale vaccination of children.

Conclusion.

Active immunisation against pertussis can be performed satisfactorily with vaccines that have proved effective in animal tests. Improved laboratory methods are now available to ensure the production of properly antigenic vaccines.

The fact that the mouse can be effectively immunised with a single

dose of a vaccine and challenged by the intranasal or intracerebral route is an important development, as it makes possible discrimination between effective and useless vaccines. Of equal importance is the knowledge of how to maintain the biological properties of *H. pertussis*, which occurs as a single serological type having characteristic growth requirements and is virulent to animals; laboratory strains differing from freshly isolated strains in serological and biological properties are artificially produced variants. The intact bacterial cell seems to give the optimal immunity response in animals; the toxic fraction of the bacterial cell is antigenic in animals, but proof is still lacking of its action in humans. Although anti-endotoxic immunity can be demonstrated in animals, the apparent absence of anti-endotoxin in convalescent and hyperimmune human sera is suggestive and counsels caution in translating laboratory evidence into medical practice. Of the diagnostic aids used (bacteriological, serological), the skin test (agglutinogen) requires further clinical trials to prove its specificity.

From laboratory experiments it is possible to draw certain conclusions to explain the failure of previous vaccines to confer immunity. First, the method of preparation of the vaccine may have been faulty; our experience shows that this is the most important reason. Unsuitable strains, deficient media, prolonged incubation, too frequent washing (especially with distilled water) of the harvested growth and, finally, too drastic killing agents—all these will affect the quality of the vaccine. From animal experiments we may conclude that the dose of vaccine, the method of administration and the spacing of the doses, depending on the type of vaccines used (plain suspension or precipitated), should be carefully considered, as these all influence the degree of protection. Reports on large-scale field trials conducted in recent years are encouraging and ampy confirm the view that immunisation against pertussis is effective with a properly prepared and tested vaccine.

Bibliography.

Barr, M., Glenny, A. T., and Randall, K. J. (1950). *Lancet*, i, 6.
Bell, J. A. (1941). *U.S. Publ. Hlth Rep.*, 56, 1535.
Bordet, J. and Gengou, O. (1906). *Ann. Inst. Pasteur*, 20, 731.
Bousfield, G. and Holt, L. B. (1947). *Lancet*, ii, 867.
Bradford, W. L. (1938). *Amer. J. Path.*, 14, 377.
Burnet, F. M. and Timmins, C. (1937). *Brit. J. Exp. Path.*, 18, 83.
Cohen, S. and Wheeler, M. (1945). *Report of Division of Laboratories and Research*, N.Y. State Dept. Hlth., p. 39.
Crawitz, G. H. and Canley, J. H. (1945). *J. Am. Med. Ass.*, 129, 541.
Demnitz, A. and Becher, H. (1939). *Mntschr. f. Kinderheilkunde*, 78, 141.
di Sant 'Agnese, P. A. (1949). *Pediatrics*, 3, 20.
Ehrich, W. E., Bondi, A., Mudd, S., and Flosdorf, E. W. (1942). *Am. J. Med. Sci.*, 204, 530.
Evans, D. G. (1940). *J. Path. Bact.*, 51, 49.
Evans, D. G. and Maitland, H. B. (1937). *Ibid.*, 45, 715.
Flosdorf, E. W., Felton, H. M., Bondi, A., and McGuinness, A. C. *Am. J. Med. Sci.*, 1943, 206, 421.
Foley, A. R. (1946). *Can. J. Publ. Hlth.*, 37, 259.
Greenberg, L. and Fleming, D. S. (1947). *Canad. J. Publ. Hlth.*, p. 279.
Harrison, W., Franklin, J. P., and Bell, J. A. (1938). *Publ. Hlth. Rep.*, 53, 793.
Holt, H. M. (1951). *Med. Officer*, p. 193.
Hornibrook, J. W. (1939). *Publ. Hlth. Rep. Wash.*, 54/2, 847.
Kendrick, P. and Eldering, G. (1936). *Am. J. Publ. Hlth.*, 26, 8.
Kendrick P., Eldering, G., Dixon, M. K., and Misver, J. (1947). *Ibid.*, 37, 803.
Kristensen, M. (1927). (*a*) *C. R. Soc. Biol.*, 96, 353.
Kristensen, M. (1922). (*b*) *Haemoglobinophilic Bacteria*, Copenhagen.

Madsen, T. (1925). *Boston M. & S. J.*, 192, 50.
Madsen, T. (1933). *J. Amer. Med. Ass.*, 101, 187.
Med. Res. Council Report (1951). *Brit. Med. J.*, i, 1464.
Miller, J. J. (1933). *Science*, 78, 340.
Miller, J. J. and Faber, H. K. (1939). *J. Amer. Med. Ass.*, 112, 1145.
Sako, W. (1947). *J. Pediatrics* (29 Jan.), 30.
Sako, W., Trenting, W. L., Witt, D. B., and Nichamin, S. J. (1945). *J. Amer. Med. Ass.*, 127, 379.
Sauer, L. W. (1935). *Am. J. Publ. Hlth.*, 11, 1226.
Sauer, L. W. (1934). *J. Amer. Med. Ass.*, 102, 1471.
Sauer, L. W. and Markley, E. D. (1946). *Ibid.*, 131, 967.
Schutze, H. (1940). *Lancet*, ii, 192.
Strean, L. P. (1940). *Can. Med. Ass. J.*, 42, 525.
Ungar, J., James, A. M., Muggleton, P. W., Pegler, H. F., and Tomich, E. G. (1950). *J. Gen. Microbiol.*, 4, 343.
Ungar, J. and Jenner, R. M. (July, 1943). *Report at Meeting of Path. Soc. G. B. & I.*, Manchester.
Ungar, J. and Muggleton, P. W. (1949). *J. Gen. Microbiol*, 3, 353.
Ungar, J. and Stevens W. K. (1951). *Ibid.*, 5, i (Proc.).
Vahlquist, B. (1949). *Lancet*, i, 16.

Discussion.

Dr. C. J. McSweeney: Few subjects are so timely and so apposite for discussion in this Section of the Royal Academy as the prophylaxis of whooping cough. Here is a disease which for scores of years has been known to be highly fatal, to be completely unresponsive to therapeutic measures, to occur in widespread and frequently-recurring epidemics in urban communities, and to leave behind in the chests of survivors, at least a predisposition to, if not a definite legacy of lung disease. Is there any medical practitioner who has not heard from the parents of tuberculous patients their conviction that the disease in the lungs dated back to an attack of whooping cough in a child who, as they put it, " has never been the same since " ? Is there anyone practising medicine for even ten years who is not forced to suspect that the chronic bronchitic and emphysematous middle-aged adult owes his disability to the devitalised or destroyed elastic tissue and the damaged bronchial mucosa produced by pertussis in childhood ? Whether or not these propositions are accepted, no one will deny that in the whole range of clinical medicine there is nothing so distressing as to watch an infant, aged a few months or even weeks, in the spasms of pertussis, getting black in the face and, for weeks on end, almost succumbing during the attacks.

Many years ago, Bordet and Gengou described and cultured a small Gram negative bacillus which since then has been regarded as the causative organism of pertussis. Based on the older ideas of vaccine prophylaxis, so notably successful in the South African War when Almroth Wright introduced the earlier vaccines against enteric infections, efforts have been made to prevent or to mitigate the ravages of whooping cough.

The story of their application to the field of medical practice has been a chequered one, and seemed to have ended when, in 1945, McFarlane, Topley and Fisher claimed to have demonstrated no difference in the incidence of pertussis in groups of inoculated and control children. I have not had the facilities to pursue a strictly scientific investigation into the merits of vaccination against whooping cough, and so I can only give the Section the experiences of nearly thirty years in inoculating children susceptible or exposed to the infection with special reference to the subsequent development of pertussis. My experience is confined to bacterial antigens not combined with others. I have never used a pertussis toxoid, alum-precipitated or otherwise. In passing, I may say that I have never believed in combined antigens. Hundreds of babies, many of them children of fellow-practitioners in Dublin, have been inoculated with vaccines of the Type I smooth faced organism during the last seventeen years. The dose varied with the particular preparation, but usually three or four injections of 0·5-1 ml. were given subcutaneously. In not one of these cases did the disease develop during the danger period for whooping cough, which is, of course, under 2 years. No one nowadays claims that even specific antigens such as we have for smallpox, diphtheria and the enteric infections confer an active immunity of longer duration than a few years. Why should the development of whooping cough at the age of 6 or 7 years in a school-child be used as an argument against infant vaccination with pertussis antigen ? Whooping cough in the pre-school age, and particularly in children of 1–2 years, is one of the most serious causes of juvenile mortality. It does not kill children over 5 years of age. If we can only postpone the age of attack, therefore, a valuable advance in preventive medicine will have been achieved. Recent work has shown that particular strains are more powerful antigenically than others previously in use. I am not in a position to compare the relative efficiency of the Glaxo, Sauer or

Kendrick pertussis vaccines. Speaking as an epidemiologist of some years' experience, I would say that the future field trials of pertussis vaccines should not be confined to any single preparation. It is a pity that it has not been possible to evolve a satisfactory intradermal test to ascertain either hypersensitiveness or susceptibility to H. pertussis. So far back as 1937, Dr. Brian O'Brien, working in my wards, using 0·1 ml. of Sauer's vaccine, found positive reactions in 71 per. cent. of whooping cough patients, and 90 per cent. negative reactions in children with no history of whooping cough. Subsequent workers, working mainly with the toxins of the organisms, have tended to confirm this work. The position, though not quite clear-cut, is in my view not very different to the results obtained in testing for susceptibility with many other bacterial antigens. Lapin (1943), in his monograph on whooping cough, concluded that "skin testing in the human must remain an unpredictable mixture of immune response and allergic response." I believe that the time is ripe for a campaign of universal immunisation of infants under six months with a single suitable pertussis antigen. Material would then be available on a considerable scale for evaluating the skin susceptibility test, first carried out in these islands by Dr. O'Brien, in addition to assessing the relative merits of different antigens. I feel sanguine that universal inoculation of infants will result in a material reduction in mortality from this disease and a diminished incidence at ages when the lung tissue is most delicate and sequelæ are most likely.

I must not conclude without some reference to certain untoward effects which have been attributed to this and other forms of immunisation in recent years. Without entering too deeply into the subject, it can be said that most of the unfavourable reactions, (e.g., encephalitis, poliomyelitis) have followed the use of *combined* antigens (e.g., pertussis and diphtheria toxoid), and many of the incriminated antigens have contained alum. In my own practice I have never, in nearly thirty years, employed a combined prophylactic or one containing alum. I am not impressed by the figures now available from the different countries as to the ætiological relationships between inoculations and diseases of the nervous system. When it is remembered how frequent and how serious is the risk of convulsions in the hundreds of thousands of cases of whooping cough which occur each year, the few cases who have been described as developing convulsions following combined antigen therapy may be discounted. Furthermore, many of the few cases described were given this combined antigen when convalescing from other viral diseases (e.g., measles), which of themselves are known to lead to encephalitis. The risk of *poliomyelitis* has been more emphatically expressed, but in our experience in Ireland, no statistically significant evidence has come to light tending to support the thesis that a child inoculated with pertussis or diphtheria antigen alone, or indeed combined, is more likely to develop poliomyelitis than a child not so injected. Far more important in our experience in determining the development and severity of clinical poliomyelitis is the degree of activity and the amount of muscular trauma to which the child is subjected after exposure to infection with polio virus. Vigorous athletic exercise of any kind, with the opportunities for trauma which this offers, is overwhelmingly more important than the prick of a needle and the local induration which a bacterial antigen may provoke. There is another relevant point in this connection. It is quite unnecessary to give antigens against diphtheria and whooping cough into a muscle. In a long experience I have never done so. I have always immunised patients against diphtheria with toxoid-antitoxin floccules, and against whooping cough with a suspension of Type I pertussis bacilli. These injections have always been given subcutaneously and have been given separately. It may have been accidental that I have never seen any untoward effects of any kind follow this avoidance of (*a*) the intra-muscular route, of (*b*) combined prophylactics, and of (*c*) alum containing products.

I believe that all infants should receive three doses of a realiable, preferably non-alum-containing pertussis vaccine. The doses are usually 1 ml. in volume, and each ml. contains 20,000 million organisms. The interval between the doses should be one month. The vaccine should be given subcutaneously, not intramuscularly. It is quite safe to begin these injections in the second month of life, but in children leading sheltered lives the injections may be postponed until the age of six months. The pertussis vaccine should be of the Type I smooth phase type and should be freshly prepared, grown from a non-mutant strain for not more than two days, and the culture should be washed in saline rather than distilled water to preserve its antigenic potency. The last war showed in the enteric infections that phenolised antigens in the prevention of enteric were inferior to suspensions treated with other reagents. If a clinician may be permitted to say so, I doubt very much whether capsulation of this indeterminate cocco-bacillus has any relation to virulence. The important thing in this new attempt to prevent one of the greatest killing diseases of infants is to procure an antigen prepared from non-mutant strains which are fresh and are not inactivated by strong antiseptics and which are not rendered locally toxic by the addition of alum.

There remains the problem of what to do with children exposed susceptibly in households, hospital wards or institutions, such as convents, orphanages, etc. There is considerable hope that an injection of hyper-immune rabbit serum given to such child-

ren with 4–5 days of exposure in a single dose of 5 c.c. may much modify the attack. But I fancy the aim of this Section of the Academy, devoted as it is to preventive medicine, will be more concerned with the active immunisation of infants and young children against this dread disease. A " booster " dose of the vaccine should be given a few years after a complete course, as in the prevention of other diseases. I am very privileged to be allowed to speak to the paper of our distinguished visitor.

Dr. HARBISON : We are investigating results, but have as yet no figures to show. I agree that if the vaccine against pertussis is pursued, the stage will be reached where pertussis will fade out. I believe the child should be immunised in the second or third month of life. Our great difficulty is to get the parents to bring the children to us.

Dr. DRUM : I know that when the Department of Health pursued an investigation in the combined pertussis and diphtheria prophylactic it was found that in the 6,000 cases treated with the combined vaccine there was no association with infantile paralysis. In 1945 we had 175 cases of diphtheria. In 1950 we had thirteen. I do not know if that was the result of vaccination.

Dr. MCSORLEY : A few years ago we tried to induce the mothers to take the combined injection. We found it difficult to get them to come to us. However, some mothers did take the combined injection, and in the last couple of years their numbers have increased. This would indicate that they realise the protection afforded their children.

Dr. FLANAGAN : Typhoid and diphtheria are more under control than they have been in the past. We hope to be able to prevent whooping cough and also measles with gamma globulin. My own experience of using whooping cough and diphtheria combined antigen has been limited. So far we have little or no data on which to base any correlation between poliomyelitis and various forms of injection in this country.

Dr. MORGAN CROWE : Has Dr. Ungar any experience of the use of the avirulent strain of the pertussis vaccine? I am mindful of the B.C.G. vaccine. Is the immunisation serum available for cases of pertussis ? Where can it be obtained ? Did the mice used in the investigation of pertussis vaccine develop the symptoms of pertussis, namely cough with spasm and a whoop ?

Dr. J. DEENY (Chairman) : We have not done any great work here yet. This disease is a killer of children. Foley's work in Canada is the only work that has had any significance. Foley immunised a number of children from one side of the river in his city and he got a concrete result. We in the profession are influenced by too many fears, whereas the parents will have the injections if they are good for the children. Gamma globulin is available against measles but is not in demand.

Dr. UNGAR (in reply) : Regarding avirulent strains. It was tried out. Once a strain is avirulent in animals we get the same degree of immunity. If a suspension is killed overnight and used straight away, the vaccine is as fully protective as a live strain. Vaccines should be used from proper strains. Some laboratories use different antiseptics. The serum is available in limited quantities. Regarding combined immunisation, a single injection is a great convenience to the child and mother, that is, both pertussis and diphtheria. Pertussis suspension is the treatment of choice in future for children. I think the animal tests are reliable. We look forward to the whole problem of controlling vaccines. In Philadelphia they immunise children against pertussis, with the result that they have not a single case of pertussis for their medical students.