common symptom. One baby developed a paroxysmal cough following the second and third injections, thought by the parents to be whooping-cough, but this was of only 24 hours' duration on each occasion.

There was no marked difference in the proportion of the total number of reactions according to the order of the three injections of fluid prophylactic in the primary course. On each occasion 379 injections were made; the total number of resulting reactions were 38, 29, and 31 respectively. The average ages at which the injections were given were 10.9, 15.5, and 20.3 weeks. The total number of reactions was least on all three occasions among the babies in group T first injected with fluid prophylactic when 6 weeks old. The proportion of babies with any kind of reaction was 4.1, 5.5, and 5.5 after successive injections. Similar figures for group V starting at 12 weeks were 12.5, 10.9, and 9.4%, and for Y starting at 16 weeks 10.7, 6.7, and 8.4%. Thus age at the time of injection is not the only factor influencing the appearance of reactions after any particular injection. Reactions to the third injection of fluid prophylactic were more frequent among those babies who had reacted before. Among the three groups together, reactions followed the third injection in 17 (5.2%) of 326 babies who had not previously reacted, and in 14 (26.4%) of 53 who had reacted before. This was specially marked in group Y: of 19 babies with reactions after the first injection, 9 (47.4%) reacted to the second and the third injection also, while 6 (3.6%) reacted out of 168 who had not reacted after either of the first two injections. General reactions were three times as frequent after injections causing local reactions than after those not followed by local reactions.

If local reactions are to be avoided when using a combined prophylactic containing pertussis vaccine given by the subcutaneous route, it is essential to use a fluid preparation.

Summary

Satisfactory diphtheria and tetanus antitoxin titres followed the use of a fluid combined diphtheria-tetanus-pertussis prophylactic.

When these injections were given at intervals of four weeks, starting at the age of 12 or 16 weeks, only one baby among 330 had a diphtheria titre below that usually regarded as the Schick level, when tested eight weeks after the third injection. Over 93% had titres at least ten times this level; over 75% had titres above 0.2 unit. Of 240 babies tested when 12 months old, none was below the Schick level and 90% had titres at least ten times this level.

Results were less satisfactory in the group receiving the first injection of the fluid preparation at the age of 6 weeks.

The adsorbed triple prophylactic caused severe reactions, and although diphtheria titres were satisfactory when the babies were 6 months old they fell rapidly during the next six months.

The tetanus antitoxin titres of all babies were above 0.1 unit per ml. on each occasion of bleeding. At the age of 6 months 95% of 460 tested had at least 1 unit per ml.

There was a rough correlation between the diphtheria and tetanus responses.

We are indebted to the staff of the child welfare department of University College Hospital for their co-operation.

REFERENCES

- Barr, M. (1954). *Proc. roy. Soc. Med.*, **47**, 352.
 — and Glenny, A. T. (1950). *Brit. J. exp. Path.*, **31**, 779.
 — and Randall, K. J. (1949). *Lancet*, **2**, 324.
 — and Llewellyn-Jones, M. (1953). *Brit. J. exp. Path.*, **34**, 12.
 Bousfield, G., and Holt, L. B. (1954). *Med. Offr.*, **92**, 289.
 Dudley, S. F., May, P. M., and O'Flynn, J. A. (1934). *Spec. Rep. Ser. med. Res. Coun. Lond.*, No. 195.
 Glenny, A. T., and Llewellyn-Jones, M. (1931). *J. Path. Bact.*, **34**, 143.
 — and Stevens, M. F. (1938). *J. roy. Army med. Cps.*, **70**, 308.

IMMUNIZATION AGAINST DIPHTHERIA AND WHOOPING-COUGH

COMBINED v. SEPARATE INOCULATIONS

BY

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During recent years increasing attention has been directed to the use of combined diphtheria-whooping-cough prophylactics. The following are the main advantages: (a) Fewer injections are needed to ensure protection against both diphtheria and whooping-cough—a matter of great importance to all concerned. (b) The use of a combined prophylactic without alum in any form, and given very early in life, may minimize the small risk of post-inoculation paralytic poliomyelitis, particularly so when administered subcutaneously. (c) A high diphtheria immunization rate is maintained: mothers demand whooping-cough immunization, but may not bother to bring their infants back for separate diphtheria inoculations (Bousfield, 1951, 1952; Bousfield and Holt, 1953, 1954).

The combined method had been in use in Barking since 1947, and continued to be employed after the "appointed day." In early 1953 the Essex County Council decided to introduce a scheme for whooping-cough immunization throughout the county. This scheme involved three injections of suspended whooping-cough vaccine, which were to be followed later by two separate injections of A.P.T. as diphtheria prophylactic. The supply of combined prophylactics was to be discontinued, presumably because of the suggestion that the development of diphtheria antitoxin might be impaired by residual maternal antibody when the prophylactic is administered in the first few months of life (Barr, Glenny, and Randall, 1950).

In view of the keen interest of one of us (V.S.) in the use of diphtheria-pertussis prophylactic—W.D.P. (red)—aroused by the report of Bousfield (1952), an approach was made to the then county medical officer, who kindly agreed that the use of combined immunization might continue in the Barking area for purposes of research. The Wright-Fleming Institute was therefore approached for a supply of their combined prophylactic—W.D.P. (red)—for the purposes of the investigation, which was from thereon planned jointly.

The following is an account of a small field trial, the main purpose of which was to ascertain whether or not satisfactory diphtheria antitoxin responses were obtained in infants following a course of combined diphtheria-pertussis prophylactic injections begun before the age of 6 months.

Strictly comparable figures—not previously available—were also provided whereby the relative efficiency of

three doses of fluid diphtheria toxoid combined with pertussis vaccine and two doses of P.T.A.P. could be compared.

The present trial gives only limited information on the relative efficiency of whooping-cough vaccines used separately and in combination with diphtheria toxoid, since the vaccines used were of different manufacture and of different *H. pertussis* content. However, the agglutination titre of the serum of each child for *H. pertussis* has been recorded, although it is not presumed, at this stage, that there is a direct correlation between agglutination titre and protection against the disease. While a number of vaccines that have given very good agglutination titres in the mouse have also given good clinical protection, the evidence for a significant correlation between the agglutinin-producing power of a vaccine and its clinical protective value awaits the publication of current M.R.C. field trials (see Evans and Perkins, 1954). The relative merits of these two courses of prophylaxis in the prevention of whooping-cough will be the subject of a later communication.

The results also enabled us to assess the degree of correlation between the response of the individual to two dissimilar antigens.

Methods

The investigation was limited to healthy babies attending infant welfare centres in Barking, whose ages ranged from 2 to 5 months inclusive on the day of the first injection. Parents of babies in this age group were approached and the objects of the investigation explained. Babies of those who did not give consent (together with those aged 6 calendar months or over) were excluded from the trial and were immunized by the separate method.

In the majority of cases parents willingly gave their consent, and the babies were then randomly allocated to separate or combined methods of injection by means of slips enclosed in sealed envelopes. Those allocated to the separate group received three injections each of 1 ml. of suspended whooping-cough vaccine (Glaxo), followed by two injections of 0.5 ml. P.T.A.P., all injections being given at monthly intervals. Babies in the combined group received three injections each of 1 ml. combined diphtheria-pertussis prophylactic W.D.P. (red), also at monthly intervals. All injections were given subcutaneously.

It should be noted that P.T.A.P. was used as diphtheria prophylactic for the separate or control group. This was done because of our previous experience of fewer and less severe reactions with P.T.A.P. than with A.P.T.

Follow-up

In the early stages it had been planned to measure the response to the diphtheria immunization by means of the Schick test at a fixed age, and that to the whooping-cough immunization by means of clinical follow-up by health visitors at regular intervals over a period of at least two years.

In the earlier trials referred to above, immunity towards diphtheria had been measured by Schick conversion rates, but the high antigenic efficiency of the reagents used was such that the limit of reliable measurement by the Schick test was reached by one dose of P.T.A.P. and by two of fluid toxoid combined with *H. pertussis* vaccine. It was thus decided in the present trial to undertake estimations of diphtheria antitoxin in the serum, and the opportunity was also taken of estimating *H. pertussis* agglutination titres.

Samples of blood for these investigations were obtained by making a small heel cut when each baby was 15 months old, irrespective of the age at which immunization was begun. Schick tests were carried out simultaneously, using B.W. Schick test toxin, but without controls. Results were read after five days.

The choice of 15 months of age for bleeding and testing was made with two considerations in mind: (a) Bousfield and Holt (1954) had found that at this age practically all unimmunized London infants are Schick-positive; and (b) the delaying effect of significant amounts of maternally bestowed antitoxin (Barr *et al.*, 1950) on the response to the course of injections would be very largely overcome by the time interval adopted (Greenberg and Fleming, 1950, 1951).

This report deals with the first 160 children from whom blood samples have been taken in this manner. Children are still being brought into the investigation, although no

TABLE I

	Males (Age in Weeks)					Females (Age in Weeks)					Total
	9-	14-	18-	22-25	All Ages	9-	14-	18-	22-25	All Ages	
Separate method ..	4	10	12	10	36	7	11	17	8	43	79
Combined method ..	10	13	11	9	43	9	7	16	6	38	81
Totals ..	14	23	23	19	79	16	18	33	14	81	160
Expected distribution..	7.5	10.25	14	8.25	40	7.5	10.25	14	8.25	40	

TABLE II.—Individual Diphtheria Antitoxin and *H. pertussis* Agglutination Titres in Infants in Barking, at Age 15 Months, Who Received Their First Injection of Combined or Separate Prophylactics at Age 2-5 Months

(a)=Diphtheria antitoxin titre, u./ml. serum.

(b)=*H. pertussis* agglutination titre (reciprocal) per ml. serum.

Combined Prophylactic 3 × 30 Lf Purified Toxoid with 10,000 million <i>H. pertussis</i> Vaccine (W.D.P.—red); 81 Cases				Separate Prophylactics 3 × 20,000 million <i>H. pertussis</i> Vaccine followed by 2 × 0.5 ml. P.T.A.P.; 79 Cases			
(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
3.0	40	0.1	40	0.6	200	0.3	300
1.25	320	0.6	160	2.0	1,280	1.25	80
1.25	160	0.4	80	0.8	400	2.0	40
1.25	640	0.5	80	1.25	20	1.25	<20
1.25	640	0.6	<20	1.25	1,000	0.8	60
0.5	40	0.4	40	2.0	320	1.5	<20
0.8	240	1.5	<20	0.8	320	1.25	250
0.9	40	0.8	<20	0.8	160	0.8	320
0.8	400	0.6	300	0.3	120	0.3	160
0.3	240	0.03	60	2.0	320	0.8	100
2.0	320	0.5	160	0.8	1,280	0.5	<20
0.2	440	0.15	60	2.0	240	1.5	80
0.3	80	3.0	80	2.0	240	0.8	320
0.8	40	0.3	160	0.8	240	0.4	<20
0.2	120	1.25	320	2.0	2,560	0.25	20
0.5	80	0.8	320	1.25	800	0.3	100
0.3	120	3.0	500	2.0	400	0.25	160
0.4	1,280	0.06	640	1.5	160	0.6	160
0.3	240	1.5	120	0.8	400	2.0	100
0.3	120	2.0	80	3.0	640	2.0	60
0.2	80	0.8	160	2.0	320	1.25	100
1.25	160	0.2	<20	1.5	1,000	0.6	60
0.2	400	0.7	80	0.8	640	2.0	80
0.8	1,280	1.7	80	0.05	1,000	0.2	300
0.5	2,560	0.5	250	0.6	400	3.0	320
0.4	240	0.06	1,000	2.0	1,000	3.0	1,280
1.0	8,000	0.3	120	0.3	640	2.0	80
0.8	6,000	0.7	25	0.125	1,280	0.5	30
0.5	1,000	0.3	100	0.6	160	0.1	160
0.5	640	0.3	<20	3.0	640	0.8	1,110
0.8	640	0.2	60	3.0	<20	0.8	120
1.25	200	0.08	<20	1.25	<20	0.8	300
2.0	160	0.04	20	0.2	20	0.8	160
3.5	<20	0.9	960	3.0	640	3.0	60
0.3	<20	0.25	<20	0.8	80	2.0	80
2.0	250	1.25	<20	0.8	40	0.025	40
0.4	40	0.3	120	1.5	20	1.0	120
0.5	<20	0.5	240	0.8	60	2.0	<20
5.0	20	0.8	3,840	1.25	160	1.0	160
0.125	20	0.8	960	0.8	120		
		1.1	240				
Geometric mean ..		0.541	109			0.883	130
S.D. log of titres ..		0.44	0.876			0.406	0.748

Statistical analyses (calculated on logs of titres)

Difference of geometric means of:

(1) Antitoxin titres, $t=3.685$, $P=<0.001$.

(2) Agglutination titres, $t=0.636$, $P=>0.5$.

Correlation between individual responses to the diphtheria prophylactic and vaccine:

(1) Separate injections, $r=0.0859$, $P=>0.4$.

(2) Combined injections, $r=0.0956$, $P=>0.4$.

TABLE III.—Comparative Field Efficiency of Three Diphtheria Immunization Procedures

	Prophylactic	Dose (ml.)	Average Age at First Injection	Interval between Injections	Average Age at Test	Units/ml. Antitoxin (Geometric Mean)	Equivalent to Schick-positive Residue per 10,000
Barr <i>et al.</i> (1950)* (61 infants)	A.P.T.	2×0.5	Approx. 20 weeks	9 weeks	8 months	0.265	30
Barking, 1954 (81 infants)	Combined vaccine (W.D.P., red)	3×1.0	18 weeks	4 "	15 "	0.54†	6
" " (79 ")	P.T.A.P.	2×0.5	32.6 weeks†	4 "	15 "	0.88‡	3

* These infants were selected—those with residual maternal antibody of more than 0.02 u./ml. being excluded.

† These infants had received separate pertussis injections × 3 commencing at average age of 18½ weeks.

‡ These results differ significantly ($P < 0.001$).

TABLE IV.—*H. Pertussis* Agglutination Titres in Infants Inoculated with Separate and Combined Prophylactics

Prophylactic	<i>H. pertussis</i> Content, Millions per ml.	Dose	Average Age at First Injection	Age at Test	Geometric Mean Titre (Reciprocal)	Percentage with Titres Below 1/20
Combined diph. pertussis (W.D.P., red)	10,000	3×1 ml.	18 weeks	15 months	109*	12.3
Separate suspended pertussis vaccine (Glaxo)	20,000	3×1 "	18½ "	15 "	130*	10.6

* These results do not differ significantly ($P = > 0.5$).

In estimating the geometric mean titre and in other calculations made on these data, titres of $< 1/20$ were taken as $1/2$.

further blood samples are being obtained. They will all be followed up clinically for a minimum period of two years, and it is hoped to present the results in a later paper.

Laboratory Estimations

The sera were assayed for diphtheria antitoxin by a micromethod based on that of Römer and Sames (1909) and for pertussis agglutination titre by using the technique and standard bacterial suspension employed by the Standards Laboratory at Colindale. Some of the first sera received were assayed against a locally prepared suspension of *H. pertussis*; the titres obtained were later adjusted by the determined ratio for the two suspensions.

Results

The 160 infants comprised 79 males and 81 females: 79 were allocated to the separate group, 81 to the combined group.

The age-sex distributions at the date of first injection are shown in Table I.

The expected age-sex distribution closely follows that observed, thus confirming the random allocation into the two groups.

At the time of entry to the trial the average age of the children in the "separate" group was 18½ weeks, that of the "combined" group 18 weeks.

Reactions.—For evidence of reactions to an injection we relied entirely on questioning parents at the time of the next injection, or (in the case of the last injection) at the time of the first follow-up visit. Minor reactions may thus have been missed, but it is worth recording that no major reactions were noted in any of these 160 children.

Schick Tests.—Of the 160 infants tested at the time of bleeding, nine were apparently Schick-positive and were expected to be brought back for retesting together with control injections. Five were shown to be pseudo-positive reactions, one (with an antitoxin titre of 0.1) was a true positive, whilst the other three did not attend. All of the five pseudo-reactors had received separate injections of P.T.A.P.

Laboratory Findings

The results obtained are shown in detail in Table II and summarized in Tables III and IV.

These results may be examined from a number of viewpoints: (1) a strict comparison of the two procedures in terms of prophylactic efficiency for diphtheria, together with comparison of these latter results with those obtained by other workers using other reagents; (2) a comparison of the two procedures so far as *H. pertussis* antibody titres are concerned; and (3), since the response towards two dis-

similar antigens was measured simultaneously, the correlation between the individual responses towards the two antigens.

Diphtheria Antitoxin Responses

Bousfield and Holt (1953, 1954) showed that the addition of vaccine to liquid toxoid considerably augmented the responses to the toxoid component of the mixture. Their field observations confirmed, in infants, the earlier laboratory findings of Greenberg and Fleming (1947, 1948) and Farago and Pusztai (1949).

It will be seen (Table II) that the geometric mean titre of diphtheria antitoxin resulting from three spaced injections of combined fluid toxoid and pertussis vaccine was 0.541 u./ml., and that from two of P.T.A.P. was 0.883 u./ml. A statistical analysis of these figures shows that the difference is highly significant ($P < 0.001$).

An alternative way to express these results is to translate them to percentage Schick-positive residue (Holt, 1955).* A geometric mean titre of 0.541 u./ml. indicates a Schick-negative rate of 99.94, or a Schick-positive residue of 6 per 10,000 infants inoculated with the combined vaccine: correspondingly a geometric mean of 0.883 u./ml. indicates a 99.97% negative rate or a positive residue of 3 per 10,000 infants inoculated with P.T.A.P.

Some comparison of our findings and the antigenic efficiency of A.P.T. in infants may be made from the data provided by Barr *et al.* (1950). Infants of average age of approximately 20 weeks possessing less than 0.02 u./ml. of antitoxin were given two 0.5-ml. doses of A.P.T. separated by an 8–10 weeks interval and bled 8–9 weeks after the second injection. The geometric mean titre of diphtheria antitoxin was 0.265/ml., which corresponds to a Schick-positive residue of about 30 per 10,000.

These results are summarized in Table III.

In any comparison with our findings the following points should be noted: (a) Barr *et al.* adopted a longer time interval than the customary four weeks between first and second injections—a procedure which, in guinea-pigs, results in a higher and more sustained antitoxin response (Barr and Llewellyn-Jones, 1951); (b) the longer time interval between the last injection and bleeding in this current trial would bias the comparison in favour of the results reported by Barr *et al.* since once the peak of the secondary response has been passed (at about two weeks) the mean titre decreases; (c) our infants were not selected for known initially low passive immunity titres; and (d) the average age at which the injections were begun in our series was 18 weeks for W.D.P. (red) and 33 weeks for the P.T.A.P. The sum total of dissimilarity between the three sets of observations would favour the antitoxin responses to A.P.T. It is clear,

*Log geometric mean = $\bar{3.5} + 0.7$ (Probit % negative – 5).

therefore, that a course of three doses of combined prophylactic (W.D.P.—red) results in a higher mean diphtheria antitoxin titre than from two—well spaced—0.5-ml. doses of A.P.T. in infants of comparable age, and that the mean titre obtained from two 0.5-ml. doses of P.T.A.P. given to infants a few months older is considerably higher. This is of interest from another point of view in that, in guinea-pigs, although one dose of P.T.A.P. results in a geometric mean some six times greater than that from A.P.T., no difference was shown when a two-dose method of assay was used (Barr and Llewellyn-Jones, 1951).

It could be argued that the antitoxin titres reported by Barr *et al.* (1950) might have been higher and more closely approaching our own results had the injections of A.P.T. been given later—a more usual age to begin separate diphtheria inoculations being 6–8 months. Their own figures suggest otherwise, since when the infants were grouped according to age at the first injection mean titres *decreased* with increase of age as follows: at age 6–13 weeks (20 cases) the geometric mean was 0.329 u./ml., at 14–26 weeks (23 cases) 0.267 u./ml., and over 26 weeks (18 cases) 0.207 u./ml.

Our findings in infants who received the combined prophylactic were different in that the older ones showed significantly higher antitoxin titres, whereas those who received P.T.A.P. (on an average 15 weeks later) showed a similar tendency, but the difference is not statistically significant. The analysis of the responses in those children who received the combined prophylactic was as follows: at ages 9–17 weeks (39 cases) a geometric mean of 0.427 u./ml., and at ages 18–25 weeks (42 cases) a geometric mean of 0.674 u./ml. ($P = 0.05$). Similarly for those who received P.T.A.P. the results were: at ages 19–32 weeks (39 cases) 0.771 u./ml., and at ages 33–42 weeks (40 cases) 1.008 u./ml. ($P = 0.2$).

The immunologist will be interested to compare responses obtained from the different antigens in children of similar ages. The medical officer of health, however, will examine these findings from a more practical point of view. It is desirable to begin whooping-cough immunization not later than the second or third month of life, and he would wish to compare results, so far as diphtheria antitoxin titres are concerned, employing (a) combined immunization starting at 2 or 3 months of age and (b) A.P.T. or P.T.A.P. separately starting at the age of 6 months. Our figures for combined immunization using W.D.P. (red) commencing before 18 weeks of age gave a geometric mean of 0.427 u./ml., equivalent to an estimated Schick-positive residue of 12 per 10,000 inoculated. Figures reported by Barr *et al.* (1950), using A.P.T. in children over 26 weeks of age, show a geometric mean of 0.207 u./ml., equivalent to an estimated Schick-positive residue of 46 per 10,000, whilst our figures, using P.T.A.P. at ages 33–42 weeks of age, give a mean of 1.008 u./ml., equivalent to a residue of 2 per 10,000.

Whilst the children given P.T.A.P. showed the higher mean titre it must be a matter of conjecture whether the additional 3–10 per 10,000 failures likely to occur if a combined prophylactic is used would be offset by the probable reduction in the number of completed courses of injections should the five-injection course (three of pertussis vaccine and two of P.T.A.P.) be adopted. We are inclined to the opinion that, since the estimated Schick-positive residue is as low as 12 per 10,000 as long as 10 months after the third injection of combined prophylactic, even with the younger babies, this itself signifies a very satisfactory immunization procedure, particularly when compared with procedures hitherto accepted as satisfactory yet yielding an estimated Schick-positive residue of 30–46 per 10,000.

H. pertussis Agglutination Responses

Our primary interest in this trial was to make an evaluation of combined and separate methods of immunizing against diphtheria. The opportunity of estimating agglutination titres to *H. pertussis* was taken, however, and these are recorded at this stage mainly for theoretical interest. Results are given in detail in Table II and summarized in Table IV.

It will be seen from Table II and the histogram that the two sets of results both show a fairly wide scatter but are virtually congruent; the geometric means (of the reciprocal) of titres are not significantly different ($P = 0.5$), and the "failure rate"—defined as the percentage of sera with an agglutination titre of $<1/20$ —is practically the same in both groups: 12.3% for the combined and 10.6% for the separate vaccine.

The number of organisms in the separate vaccine was 20,000 million per ml., but only 10,000 million per ml. in the combined vaccine; since, however, the vaccines were of different manufactures it is not possible to say whether: (a) the antigenic power of the organisms contained in the

Distribution of agglutination titres, for *H. pertussis*, in sera of children who received separate and combined immunization.

separate vaccine was less than that in the combined vaccine; (b) the diphtheria toxoid potentiated the action of the pertussis antigen in the combined vaccine; (c) the P.T.A.P. given subsequently interfered with the response to the separate pertussis vaccine; or (d) an effective "ceiling" response can be obtained with a vaccine containing only 10,000 million organisms per ml. The possibility of there being an optimum inoculation is the subject of a further clinical trial.

Inoculation with the combined vaccine, containing only half the number of *H. pertussis* organisms, resulted in titres not significantly below those achieved with the separate pertussis vaccine. Combined diphtheria pertussis prophylactic—W.D.P. (red)—now contains 20,000 million organisms per ml., and it thus seems reasonable to suppose that inoculation with this prophylactic will yield agglutination titres at least as high as, if not higher than, those resulting from the use of separate suspended pertussis vaccine (Glaxo).

We do not yet know whether there will be any significant difference between the two vaccines so far as protection against whooping-cough is concerned. All that can be said at this stage is that our results give no reason to suppose that a pertussis vaccine combined with fluid diphtheria toxoid is any less efficient antigenically than one given separately.

Correlation Between Individual Responses to More Than One Type of Antigen

That an individual may respond well to one class of antigen and poorly to another has been suspected for some time. Carlinfant (1948), writing on this subject, states: "There does exist a correlation among the titres of antibodies produced by one subject in response to different antigens (of the same class). . . . Antibacterial somatic antibodies are correlated with flagellar ones, diphtheria antitoxin with the tetanal one . . . but there does not seem to exist a correlation or at least one so well marked between haemagglutinins, antitoxins, and antibacterial antibodies."

The above body of data provided excellent material whereby the degree of correlation between the responses of infants to two very dissimilar antigens could be calculated.

Calculations with combined and separate groups in both cases give a correlation coefficient r of <0.1 and P of >0.4 . (Since the distribution of responses to both antigens is log normal all calculations were made on logs of titres.) This signifies a complete absence of relationship between the human infant's responsiveness to diphtheria toxoid and to the agglutinin of *H. pertussis* vaccine.

Summary

Two similar groups of urban infants (Barking, Essex) aged from 2 to 5 months inclusive at time of first injection were immunized against diphtheria and whooping-cough in different ways: (a) A course of three injections of combined prophylactic (W.D.P.—red). (b) A separate course of five injections: the course of three injections of suspended pertussis vaccine (Glaxo) preceding that of two doses of diphtheria prophylactic P.T.A.P. All injections were made at monthly intervals, each child was bled at 15 months of age, and the sera were assayed for their diphtheria antitoxin and *H. pertussis* agglutination titres.

Three doses of the combined prophylactic—W.D.P. (red)—gave significantly higher antitoxin titres than those previously reported for two doses of A.P.T. It is noteworthy that the very young infants inoculated with the combined prophylactic gave higher antitoxin titres than somewhat older ones given A.P.T. Two doses of P.T.A.P., however, gave the highest figures.

Although whooping-cough vaccines of different manufacture and *H. pertussis* content were used in the two groups the titres obtained were of the same order, and showed no significant difference.

No correlation was found between the responses of individuals to the diphtheria toxoid and the *H. pertussis* agglutinin.

We gratefully acknowledge the help of other medical officers who assisted with the injections, and that of the health visitors in persuading parents to include their children in the research, as well as for the important part they are playing in the clinical follow-up. We have received invaluable assistance from Mr. Brian in the collection of blood specimens, and a considerable amount of clerical assistance from Mrs. Joan Saul. Thanks are also due to Mrs. Ann Watts for her help in the serum titrations.

REFERENCES

- Barr, M., Glenn, A. T., and Randall, K. J. (1950). *Lancet*, **1**, 6.
 — and Llewellyn-Jones, M. (1951). *Brit. J. exp. Path.*, **32**, 231.
 Bousfield, G. (1951). *Lancet*, **1**, 1028.
 — (1952). *Med. Offr.*, **87**, 265.
 — and Holt, L. B. (1953). VI Int. Congr. Microbiol., Rome. Comm. No. 307.
 — (1954). *Med. Offr.*, **92**, 289.
 Carlinfanti, E. (1948). *J. Immunol.*, **59**, 1.
 Evans, D. G., and Perkins, F. T. (1954). *J. Path. Bact.*, **68**, 251.
 Farago, F., and Pusztai, S. (1949). *Brit. J. exp. Path.*, **30**, 572.
 Greenberg, L., and Fleming, D. S. (1947). *Canad. J. publ. Hlth.*, **38**, 279.
 — (1948). *Ibid.*, **39**, 131.
 — (1950). *J. Pediat.*, **36**, 143.
 — (1951). *Ibid.*, **39**, 672.
 Holt, L. B. (1955). *Biometrics*, **11**, 83.
 Römer, P. H., and Sames, Th. (1909). *Z. Immunforsch.*, **3**, 344.

The Department of Public Health of the Territory of Papua and New Guinea published last May the first number of the *Papua and New Guinea Medical Journal*. It is to appear quarterly. The Director of the Department, J. T. GUNTHER, explains in his foreword: "The journal is intended to keep all our varied grades of medical personnel abreast of world progress in the prevention and treatment of the disease pattern of the Territory, to promote medical research locally into these diseases, and to facilitate an exchange of ideas between officers who are otherwise geographically isolated." The first issue includes original articles, extracts from current literature, clinical notes, and book reviews.

THE RELATIONSHIP OF THE ABO BLOOD GROUPS TO DUODENAL AND GASTRIC ULCERATION

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This investigation concerns the distribution of the ABO blood groups in 1,665 duodenal and gastric ulcer patients drawn from three Liverpool Hospitals. It was prompted by the recent paper by Aird *et al.* (1954) in which they showed a greatly increased incidence of blood Group O in 3,011 patients with peptic ulcer from London, Manchester, and Newcastle. The higher frequency of Group O in their series was more marked in duodenal than in gastric ulcer, but the difference between the two sites was not significant on the numbers available. Their overall finding was that persons of Group O are about 35% more likely to develop peptic ulceration than are persons of the other groups.

Collection of Data

The three Liverpool hospitals in which the survey was carried out were the Liverpool Royal Infirmary (374 beds), the David Lewis Northern Hospital (208 beds), and Broadgreen Hospital (657 beds). The first two of these belong to the teaching group and the third to the Regional Board.

The Royal Infirmary ulcer cases were obtained by two of us (W. H. E. and J. C. W.) from an examination of the disease index and operating theatre record books between the years 1948 and 1954. The total number of patients (5,109) who had been blood-grouped in the hospital during 1949–54 inclusive was also recorded.

In the David Lewis Northern Hospital the same two members of the team (W. H. E. and J. C. W.) first extracted from the pathological records the names of the 3,257 patients who, irrespective of diagnosis, had been blood-grouped during the years 1948–54. From this source and from the disease index the case sheets of all patients diagnosed as having had duodenal or gastric ulceration were obtained.

In Broadgreen Hospital two of us (J. W. E. and W. K. C.) scrutinized the pathological records and from them extracted the case sheets of the 8,706 patients who had been blood-grouped during the years 1948–54. From this total were obtained the notes of all the ulcer patients.

Criteria of Diagnosis and Classification of Material

In all three hospitals the diagnosis of ulceration had to be supported either by macroscopical evidence—operation findings, gastroscopy report, or necropsy material—or by a definite positive radiological opinion. Those diagnosed macroscopically are recorded separately from those in which the diagnosis was only radiological. There were very few of the latter except at Broadgreen Hospital, which, as a regional hospital, admits many emergency cases of haemat-