

**SEROVERSION IN URBAN AND RURAL POPULATIONS
OF CHILDREN FOLLOWING DOSES OF TRIPLE ANTIGEN**

**DISSERTATION SUBMITTED FOR THE DEGREE OF
DOCTOR OF MEDICINE IN MICROBIOLOGY**

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INTRODUCTION

Diphtheria-Pertussis-Tetanus :

In 1888 Roux and Yersin at the Institut Pasteur in Paris demonstrated that the virulence of C.diphtheriae was chiefly due to the production of a soluble toxin. Based on this knowledge, the antitoxin was soon discovered and successfully used, in 1891, for treatment. In 1898, Salkowski described a method of modification of the toxin with formalin and such a toxoid was found to retain its antigenicity without demonstrating any toxicity. However, the actual use of the toxoid for active immunization commenced in 1923 and the actual preparation used was a toxin-antitoxin mixture. The conquest of diphtheria by active immunization is one of the outstanding triumphs of preventive medicine. In many of the economically developing countries, where diphtheria inoculation has been fully practised, deaths from diphtheria are now negligible, whereas, in the 1930s, diphtheria was the commonest cause of death amongst school-going children. In these developing countries, however, mass immunization against diphtheria is not yet compulsory, nor customary and the disease is still endemic and an important cause of morbidity and death.

The first certain description of Bordetella pertussis was by Bordet of the Institut Pasteur, in Brussels, who,

in 1900, visualized the causative organisms of whooping cough in the sputum of his infant daughter. Subsequently, six years later, he successfully cultured the organism along with Gengou. Soon after the isolation of the causative agent, Bordetella pertussis, attempts were made to prepare a vaccine for active immunization. Since a clinical attack of whooping cough gives rise to long lasting immunity, high hopes were held for successful vaccination. However, due to various technical reasons, immunization against pertussis proved very difficult in the beginning and, it is only since 1946, that an effective vaccine has become available. A substantial reduction in pertussis infection has been achieved in the economically developed nations, since 1955, following the introduction of nation-wide immunization programmes. However, even the debatable efficacy of pertussis immunization still outweighs the well-recognized side-reactions associated with the vaccine.

In 1889, Kitasato discovered Clostridium tetani as the aetiological agent of tetanus and, in 1890, von Behring and Kitasato demonstrated the filterable tetanus toxin, to which the organism owes its virulence. Soon afterwards, the protective action of tetanus antitoxin was established by the same workers and passive prophylaxis commenced with horse antitoxin. However, it was not until 1924, when Descomby produced tetanus toxoid

by formalinization of toxin. In 1927, Zoeller and Ramon of the Institut Pasteur, Paris, used tetanus toxoid, for the first time, for immunization of human volunteers.

Since the efficacy of antitetanus immunization was amply demonstrated in the Second World War, it appeared that active immunization could eradicate the disease, if applied systematically to entire populations.

Combined vaccine (DPT, Triple Antigen) :

Castellani was the first to produce a mixture of these antigens, but most of his work was actually carried out with mixed typhoid and paratyphoid A and B vaccines, which are of similar antigenic constitution and it was Ramon, in 1926, who first mixed a toxoid with bacterial antigen. In 1940, the administration of mixed tetanus and diphtheria toxoids was made compulsory, for all children reaching one year of age, in France.

Bordet (1936) suggested that diphtheria toxoid could be added to pertussis vaccine and a similar suggestion was made by Ledingham, in 1938.

The advantages of effectively mixing diphtheria, pertussis and tetanus antigens include reduction in the

number of injections, with a consequent simplification of administrative problems and the reduction in the expenditure involved in mass campaigns. A further advantage claimed is that the immunizing potency of the toxoid is increased, without interference with the immunogenic capacity of the vaccine.

DPT vaccine :

The combination of diphtheria and tetanus toxoids and pertussis vaccine, commonly known as triple vaccine (or, triple antigen, DTP, or DPT vaccine), is the most useful preparation for primary immunization of infants and has been accepted as a routine public health measure in many countries. The plain, or unadsorbed, DPT vaccine has been replaced by a more potent adsorbed vaccine practically around the globe. Usually, aluminium hydroxide, or aluminium phosphate is utilized for adsorption; however, some manufacturers still continue to use the less effective alum for this purpose. Each of the three components is separately prepared and then adsorbed on to the aluminium salt and blended together. A commonly used formula contains, in a 0.5 ml dose, E. pertussis 20,000 million organisms (potency 4 International Units), purified diphtheria toxoid 25 Lf purified tetanus toxoid 5 Lf, adsorbed on aluminium

hydroxide, or phosphate and thiomersal 0.01 per cent, as preservative. The quantities of each component are so balanced that there is no interference and maximum benefit is obtained. Adsorption on aluminium hydroxide has been found not only to increase the potency but, also, to reduce the tendency of the vaccine to cause reactions.

Immunization schedule for
Triple Antigen administration :

A three dose immunization schedule with DPT vaccine given at intervals of one, or two, months is generally considered necessary to obtain a satisfactory serologic response against the three components of the vaccine. On the basis of the work of Miller et al. (1947) and Sato (1947), it is assumed that an agglutination response of 1/320 is an indication of clinical immunity against a pertussis fraction. In addition, in the British field trials (M.R.C. 1956), there was, in general, a correlation between protection in the field and agglutinin response, in children. For diphtheria and tetanus, a level of 0.01-0.03 antitoxin titre per ml serum has generally been accepted as necessary to confer complete protection from the toxic manifestations of the infections (Newell et al., 1971; Ypsen, 1946). Miller, Humber and

Dawrie injected children of ages between 6-12 months with triple antigen giving two doses, at three-monthly intervals. Each dose contained 26-30 Lf adsorbed diphtheria toxoid, 100,000-120,000 MLD adsorbed tetanus toxoid and 20,000 million B.pertussis. Six months after the second dose, all of 114 children tested were Schick-negative and 65 % of 37 children had tetanus antitoxin titres exceeding one unit/ml and all had more than 0.01 unit level which is considered indicative of protection.

Peterson and Christie began a study, in 1944, in children from 4 weeks of age upwards. Some 2000 children were inoculated and 573 were followed up for thirteen months. Three injections at 6-weekly intervals were given. The first and third contained adsorbed diphtheria toxoid (30 Lf), adsorbed tetanus toxoid (100,000 MLD) and adsorbed pertussis vaccine, 20,000 million organisms per ml. The second dose consisted of 40,000 million B.pertussis adsorbed vaccine. The titres were satisfactory in children over 6 months of age at the time of the first inoculation, but were less satisfactory in children under 6 months. The tetanus antitoxin titres were satisfactory at all ages. Ninety-six per cent of 573 children had titres greater than 0.1 unit, at three months after investigation and 98 % had similar titres 13 months post-immunization.

Expanded Immunization Programme :

In recent years, much attention has been given by the WHO in its Expanded Immunization Programme, to the problems connected with immunization against diphtheria, pertussis and tetanus in the economically developing countries. In these countries, the traditional schedule, comprising three injections of DPT vaccine, given at intervals of one, or two months is often applied. In view of the fact that the infrastructure of health facilities in these countries is often weak, that there are long distances to be covered by the parents desirous to get their children vaccinated or the vaccination team and that the population migrates, it usually upsets the schedule of timely placed boosters. The resultant, thereof, is an inadequately immunized child population, almost equally susceptible to the disease, to which the unimmunized population usually succumbs.

Perkins (1977) has pointed out that there are several points at which shipment and distribution of vaccine may be protracted. From the observation of Stainer and Hart (1978), it is clear that the bacterial antigens are relatively stable even under such extreme conditions, as storage in the bulk (or filled form) at 37°C for extended periods. Pertussis vaccine has

been criticized in recent years and several authors have questioned its efficacy, safety and stability (Bassili et al., 1976; Dick, 1974; Stewart, 1977). However, it is evident from the data presented that even this vaccine retains its potency for a reasonable length of time at 24°C or 37°C. It might be tempting, in view of this evidence, to conclude that the temperature of storage for bacterial vaccine is not critical within fairly wide limits. This is certainly not the case. Vaccine should be stored at the temperatures recommended by the manufacturers and, for the immunization programme to be effective, strict control should be maintained over vaccine manufacture, testing, distribution and methodology of use.

It is clear that with adsorbed diphtheria and tetanus toxoids, added to plain or adsorbed pertussis vaccine, a satisfactory level for diphtheria antitoxin can be obtained, at least in children over 6 months of age. With tetanus toxoid, satisfactory levels can be obtained at any age and, as far as the evidence goes, it does not appear that mixing the toxoids interferes with antitoxic responses to each of the antigens in children who are being primarily immunized.

There is tremendous need for large quanta of combined vaccines, such as triple vaccine, in economically developing countries such as ours, with their

enormous child populations. Even if 70% of the children are to be covered, the annual requirement in India would be about 70 million doses of DPT vaccine, while the production capacity, as it stands today, is merely 20 million !

AIMS AND OBJECTIVES

Thus, although there is reasonably good evidence that mixed antigens are useful in immunization campaigns, extensive, carefully-controlled investigations must still be undertaken in man and animals, before their value can be finally assessed. Studies are required on the problem of interference, on the duration of immunity with mixed and separate antigens, on the relative amounts of antigen required to produce balanced antibody responses and on posology and the spacing of doses for primary immunization. In particular, further information is required regarding time-intervals, optimal reinforcing doses and on the importance of interference with naturally-acquired, passively-transferred antibodies from mother to infants and primary immunization with mixed antigens used for the purpose. Though many of these studies can be conducted by way of serological testing, further epidemiological studies, along with the field trials, are mandatory for correct assessment of the efficacy of antigens utilized.

The necessity of properly-designed field trials for the evaluation of vaccines was fully recognised by Haffkine, in the 19th century. He was in fact, the first scientist to define the principles of controlled

field investigations and he applied these during his trials on cholera and plague vaccines, in our country.

In many economically developing countries, vaccines are extensively used or advocated, without ascertaining their efficacy in the regional context. It is recognised that the controlled field trial of a vaccine constitutes a sound scientific basis which correctly assesses the efficacy of that vaccine and thus avoids continuation, or fresh introduction of worthless preparations.

A criterion for the assessment of protection afforded by a vaccine is whether it causes a reduction in the incidence of the particular disease, or enhances the level of those antibodies which have been shown, by immunological tests, to be positively associated with protection.

Although adequately vaccinated, some children develop the disease against which they have been, presumably, immunized. Vaccine failure, therefore, affects the acceptability of immunization as much as its complications and is a cause of considerable concern in mass immunization programmes. However, serological surveys for seroconversion in the vaccinated groups, can ensure whether it is, indeed, the vaccination which has failed.

Most of the rural child population in commercially developing countries like ours, suffers from a mild to

moderate degree of undernutrition. Apart from advocating the improvement in dietetic programmes, it is also important to protect these children from diseases, which may show an escalated picture in those of poor nutritional status. Once again, one wonders whether the immunological responses in such children are altered, aberrant, or nearly absent, which could account for vaccine failures. In order to assess such differential responses towards vaccination, it is essential to conduct trials of common vaccines in the context of rural areas in comparison with the urbanised communities.

Such thoughts as these, prompted the present author to conduct controlled field trials of commonly employed vaccines in rural and urban communities. The results of immunization with cholera vaccines, in individuals of both sexes from these communities, as well as maternal immunization against tetanus have been presented by us earlier (Chanderkar, N.G., Ph.D. Thesis, 1978). The present dissertation outlines the data obtained from a trial conducted in a limited number of children from rural and urban areas, using triple antigen prepared at the Haffkine Institute, Bombay.

MATERIALS AND METHODS

Serum samples :

Approximately 3-5 ml of blood was collected from each child through femoral venipuncture with aseptic precautions. The sera were separated and merthiolate added to a final concentration of 1:10,000 as a preservative. All sera were stored at -20°C till use. At the first visit, for purposes of immunization, attempts were made to elicit the history, in each case, regarding earlier vaccination, or an episode of sore throat and whooping cough, even though the information thus collected was not solely relied upon in the final analysis of data.

Repeated sampling of the same vaccines was attempted for most of the cases, even though the interval between two successive doses might have been a little longer than that indicated for the majority. Thus, the samples which are labelled 1st to 4th are as follows:

- 1 Basal (Pre-immunization), just prior to administration of the first dose of DPT.
- 2 6-8 weeks after the first dose and just prior to 2nd.
- 3 6-8 weeks after the second dose and just prior to 3rd.
- 4 4-6 weeks after the 3rd dose.

The two groups of children were from two different ecosystems viz. from urban and rural situations, of variable nutritional, socioeconomic and ecological status:

- Group 1 Children from urban areas,
 attending the Child Care
 Centre, H.H.Sayedna Saifuddin
 Trust, Bombay.
- Group 2 Children from rural area,
 attending the Primary Health
 Centre, Virar, District Thane,
 Maharashtra.

The number of samples from each of these groups at each dose-interval are presented under Results (Table 1).

Haemagglutination test for
human anti-tetanus and
antidiphtheria antibody
quantification :

Principle.

Erythrocytes were coated with antigen after treatment with tannic acid. Such coated cells were used as markers in a haemagglutination reaction with specific antisera. Thus, the antibody titres of sera were assessed by a standard titration method.

Reagents.

- 1 Standard purified tetanus and diphtheria and antitoxin. The same batches were used in all tests.
- 2 Normal saline (0.85 % NaCl) phosphate-buffered saline (PBS pH 6.4 and 7.2).
- 3 Tannic acid (analytical grade).
- 4 Normal rabbit serum.

Procedure.

Human group O erythrocytes collected in ACD solution were washed twice with normal saline and once with PBS (pH 7.2), resuspended in PBS (pH 7.2) and washed with PBS (pH 6.4) to remove traces of the tannic acid. They were further treated with either tetanus, or diphtheria toxoid, diluted in PBS (pH 6.4) and containing 35-40 μ g/ml. Finally, they were washed twice and resuspended in PBS (pH 6.4) with 1 % normal rabbit serum, being then ready for use.

Standard antitoxin was serially diluted two-fold until the concentration of antitoxin per well of the haemagglutination tray was 0.0002 IU/ml. The test sera

were similarly diluted. The comparison between the end point dilution of the standard to that of the test serum yielded the quantum of antitoxin present in the latter.

Antipertussis antibodies :

For preparation of pertussis antigen Strain No. 509 was used. The strain was grown at 37 °C in Verwey medium by incubation for 40-48 h. The cells were harvested by centrifugation at 2000 rpm for 1 h and resuspended in normal saline. Optical density was adjusted to around 150×10^9 cells/ml. The organisms were heat-inactivated at 56 °C for 15 min. Merthiolate was used as a preservative and the preparation stored at 4 °C. A suspension of final opacity of 20×10^9 cells/ml was used in the agglutination test. The agglutination test was performed as per the recommendations of the WHO. The results were expressed as titre of antibodies. A titre of 1/320 and above is generally accepted as an adequate protective titre (Miller and Sako, 1947).

RESULTS

Agewise distribution of children belonging to the two groups has been presented in Table 1. These study groups differed in terms of geographical area and socioeconomic status. Thus, group 1 (Urban) consisted of 268 children, of either sex, in different age range, attending an immunization clinic in an urban area (Bombay City), while group 2, comprising 307 children was immunized at a Primary Health Centre in a rural area, (Virar, District Thane), situated about 50 km North-East of Bombay City.

It may be noted that 70.1 % of the children from the urban area were among the prescribed (for primary immunization) age-range 3-5 months, while only 4.4 % were above 12 months of age. In contrast to this situation, in the rural area, 29 % of the children were in the prescribed age-range and nearly 19.4 % were above 12 months when they presented themselves for the first dose of DPT immunization.

More than 45 % of children from an urban area completed the three-dose schedule of DPT immunisation in time. Eighty per cent took the second dose. In the rural area, however, only 45 % presented themselves for the second dose, while only 25 % took the third dose, thus completing the schedule.

Pre-immunization antibody levels :

Tetanus.

Table 2 presents the pre-immunization antitetanus antibody levels in the two groups of children, at various age-range subgroups. In the urban area, 15.2 % of the children showed antitetanus antibody levels above protective range of 0.01 IU/ml. 77.2 % however did not possess any evidence of antibodies against tetanus. In the rural situation, 91 % did not exhibit any antitetanus antibodies and only 4.6 % were above the protective range. Thus, there was significant difference in the antibody number of children from two groups having basal antitetanus antibody levels above detectable or protective limit.

More than 50 % of the children from the urban area showing antibody levels above the protective range were in the age-range 3-5 months and only 8 % were in the age-range over 9 months. Similarly, higher levels of antibodies were found in the age-range 3-6 months and none of the children over 24 months of age showed the presence of any detectable level of antitetanus antibodies.

As compared to this group only 2.9 % of the children in the age range of 3-6 months from rural area showed protective or detectable levels of antitetanus antibodies.

Diphtheria.

Table 3 shows the pre-immunization antidiphtheria antibody levels in the two groups of children. As in the case of tetanus, only 23 % of, urban as well 36 % of rural, children showed the presence of antidiphtheria antibodies, with only 14 % and 24 % respectively, from both urban and rural groups being above the protective range. In contrast to tetanus, however, older children in the range of 6 months and above showed antidiphtheria antibodies in higher titres.

Pertussis.

The antipertussis antibody profile prior to immunization in the two groups of children at various age-ranges has been presented in Table 4. In contrast to the other two diseases mentioned above, the children in the younger age-ranges, from both the groups were categorily devoid of any antipertussis antibodies, only 8 % showing titres between 1/20 - 1/40 while only one from rural area was in the protective range. In the higher age-range, between 12-24 months, there were 7 individuals with antipertussis antibodies, titre more than 1/40 from the rural set-up, as against only 3 individuals from urban area.

Table 5 presents a composite picture of pre-immunization antibody levels against diphtheria, tetanus and pertussis in the two groups of children. Amongst these three diseases, preimmunized antibody levels against pertussis were evident in 50-60 % of children, next in order being tetanus and, lastly, diphtheria. For diphtheria, the number of children possessing protective levels of antibody were 14.1 % and 7.1 %, respectively, for urban and rural areas, while only 15.2 % and 4.8 % were within protective limits for tetanus. In case of pertussis, however, only a single child from the rural area showed antibody over the protective range (1/160).

Post-immunization antibody levels :

Tetanus.

Table 6 shows the periodically-estimated antitetanus antibody levels following one to three doses of triple antigen in the two groups of children. Initially, at the basal (pre-immunization) level, there was a significant difference in the number of children between the two groups possessing pre-immunization protective levels. 14.9 % of the children from the urban area had protective antibody levels, as against only 4.8 % in the rural area. The response towards the immunization regimen was, however, quite similar in both the groups:

in the urban, 68.8 % of the children were covered under the protective titre of 0.01 IU/ml and over, as against 60.0 % in the rural group, following two doses of antigen. The figures following the 3rd dose of the regimen were 90.0 and 86.0 %, respectively. In actuality, however, as there were many drop-outs during the course of immunization, conclusions can best be derived only from a consideration of the basal (pre-immunization) antibody status of the total numbers of children taking part in the study of the two groups viz. 268 and 307, respectively.

Diphtheria.

The antidiphtheria antibody levels following DPT immunization in the two groups of children are presented in Table 7. Once again, as in the case of tetanus, the percentage of City children showing basal antibody levels in excess of the protective range was nearly twice that of the rural group (14.1 % versus 7.1 %), whereas the subsequent response at the 2nd and 3rd doses was almost similar in both groups: thus, the protective level figures, after the 2nd and 3rd doses for the urban and rural groups were, respectively, 61.6 % and 57.5 %, versus 80.0 % and 76.0 %. The antibody levels were determined 15-20 days following completion of the

immunization regimen. Thus, there was no significant difference between the 2 groups, as apparent on inspection.

Pertussis.

The pre- and post-immunization antipertussis antibody levels from the two groups are presented in Table 8. As compared to the tetanus and diphtheria fractions of the combined DPT vaccine, the response towards pertussis was not as pronounced, since, in spite of receiving two doses of vaccine, only 24.0 % of the children from the urban and 25 % from the rural area exhibited antibody titres of over 1/40. However, following the third dose of vaccine, only 13.7 % and 28 % of the children from both the urban and rural groups did not develop any pertussis antibodies while 32.5 % and 28.0 %, respectively, displayed antibody levels exceeding 1/160 and were approaching the protective limit.

Similar to pre-immunization status Table 9 presents a composite temporal profile of the development of antibodies against diphtheria, pertussis and tetanus immunization schedule in response to the immunizing 3-dose regimen. It shows comparable and most significant elevation of specific antibody levels against the above mentioned diseases, in both groups of children.

Although only a small number of children had, prior to immunization, protective levels of antibody levels, it must be noted that almost 90 % of them showed a favourable response towards the tetanus and diphtheria fractions of the triple antigen. As many as 20 % of the children from both the groups had shown antitetanus and antidiphtheria antibody levels approaching even upto 1.0 IU/ml following three doses of antigen. The pertussis fraction, however, did not act similarly, the antibody titres in children, even following 3 doses, being comparatively low.

Comparative efficacy of the three components of DPT vaccine :

Table 10 summarises the comparative efficacy of each of the 3 components of triple antigen following each of the 3 doses at monthly intervals. It can be noted from this table that tetanus toxoid proved to be statistically significant superior antigenic material at each dose level as compared to diphtheria toxoid which was next in superiority. The pertussis fraction was, however, low in antigenicity, especially when only two doses were given.

Thus, the data from Table 10 indicate that, for optimal immunization, it is essential to administer

3 doses of DPT at 4-6 weeks intervals, even two doses being inadequate for coverage of the susceptible community in mass immunization campaigns.

Table 1

AGEWISE DISTRIBUTION OF CHILDREN FROM URBAN
AND RURAL AREAS IMMUNIZED WITH TRIPLE ANTIGEN

Group	State of immunization dose	Number of samples										Total	
		Age (Months)											
		3	-	5	6	-	8	9	-	11	Above 12		Above 24
1 (Urban)	Basal	188 (70.1)		41 (15.2)				27 (10.0)		9 (3.3)		3 (1.1)	268
	1st	81 (38.0)		101 (47.4)				22 (10.3)		8 (3.7)		1 (0.4)	213 (79.4)
	2nd	-		103 (82.4)				19 (15.2)		2 (1.6)		1 (0.8)	125 (46.6)
	3rd	-		66 (82.5)				12 (15.0)		11 (13.75)		1 (1.2)	80 (29.0)
2 (Rural)	Basal	89 (28.9)		102 (33.2)				56 (18.2)		41 (13.3)		19 (6.1)	307
	1st	52 (36.8)		61 (43.2)				19 (13.4)		8 (5.6)		1 (0.70)	141 (45.9)
	2nd	31 (38.7)		41 (41.2)				6 (7.5)		1 (1.25)		1 (1.2)	80 (26.05)
	3rd	-		23 (46.0)				24 (48.0)		2 (4.0)		1 (2.0)	50 (16.2)

Figures in parentheses indicate percentages

Table 2
PRE-IMMUNIZATION ANTITETANUS ANTIBODY LEVELS IN
VARIOUS AGE GROUPS OF CHILDREN FROM TWO DIFFERENT AREAS

Antitoxin level (IU/ml)	Number of children in age range (months)										Total No. and percentage of children				
	3	-	5	6	-	8	9	-	11	Over 12			Over 24		
	U		R	U		R	U		R	U	R	U	R		
< 0.001	146 (70.5)		79 (28.1)	30 (14.4)		92 (32.7)	21 (7.4)		52 (18.5)	7 (3.3)	39 (13.8)	3 (1.4)	19 (6.76)	207 (77.2)	281 (91.5)
> 0.001-<0.01	14 (70.0)		5 (45.4)	4 (20.0)		4 (36.3)	1 (5.0)		1 (9.0)	1 (5.0)	1 (9.0)	-	-	20 (7.4)	11 (3.58)
> 0.01-<0.05	14 (63.6)		3 (37.5)	6 (27.2)		2 (25.0)	1 (4.5)		2 (25.0)	1 (4.5)	1 (12.5)	-	-	22 (8.2)	8 (2.60)
> 0.05-<0.1	14 (73.6)		2 (28.5)	1 (5.2)		4 (57.1)	4 (21.0)		1 (14.2)	-	-	-	-	19 (7.0)	7 (2.2)
n =	188 (70.1)		89 (28.9)	41 (15.2)		102 (33.2)	27 (10.0)		56 (18.2)	9 (3.3)	41 (13.3)	3 (1.1)	19 (6.1)	268	307

Figures in parentheses indicate percentages

U = Urban

R = Rural

n = Total number of children

Table 3

PRE-IMMUNIZATION ANTIDIPHTHERIA ANTIBODY LEVELS IN
VARIOUS AGE GROUPS OF CHILDREN FROM TWO DIFFERENT AREAS

Antitoxin level (IU/ml)	Number of children in age range (months)												Total No. and percentage of children		
	3	-	5	6	-	8	9	-	11	Over 12	Over 24				
	U		R	U		R	U		R	U	R	U	R		
< 0.001	149 (73.7)		67 (26.9)	25 (12.3)		86 (34.5)	20 (9.9)		49 (19.6)	6 (2.9)	35 (14.0)	2 (0.9)	12 (4.8)	202 (75.3)	249 (81.1)
> 0.001-<0.01	17 (60.7)		13 (36.1)	4 (14.2)		9 (25.0)	4 (14.2)		6 (16.6)	2 (7.1)	3 (8.3)	1 (3.5)	5 (13.8)	28 (10.4)	36 (11.7)
> 0.01-<0.05	15 (71.4)		6 (37.5)	3 (14.2)		5 (31.2)	2 (9.5)		1 (6.25)	1 (4.7)	2 (12.5)	-	2	21 (7.8)	16 (5.21)
> 0.05-<0.1	7 (41.1)		3 (50.0)	9 (52.9)		2 (33.3)	1 (5.9)		-	-	1 (16.6)	-	-	17 (6.3)	6 (19.5)
n =	188 (70.1)		89 (28.9)	41 (15.2)		102 (33.2)	27 (10.0)		56 (18.2)	9 (3.3)	41 (13.3)	3 (1.11)	19 (6.1)	268	307

Figures in parentheses indicate percentages

U = Urban

R = Rural

n = Total number of children

Table 4
PRE-IMMUNIZATION ANTIPERTUSSIS ANTIBODY LEVELS IN VARIOUS
AGE GROUPS OF CHILDREN FROM TWO DIFFERENT AREAS

Antipertussis Antibodies Titre	Number of children in the age range (months)												Total No. and percentage of children		
	3	-	5	6	-	8	9	-	11	Over 12	Over 24			U	R
	U		R	U		R	U		R	U	R	U	R		
1/10	90 (80.3)		64 (62.1)	16 (14.2)		21 (20.3)	5 (4.46)		13 (12.6)	1 (0.89)	3 (2.9)	-	2 (1.9)	112 (41.7)	103 (33.5)
1/10- 1/20	98 (71.5)		18 (10.2)	23 (16.7)		73 (41.4)	10 (7.2)		41 (23.2)	4 (2.9)	30 (17.0)	2 (1.4)	14 (7.9)	137 (51.1)	176 (57.3)
1/20- 1/40	-		6 (46.1)	2 (15.3)		2 (15.3)	9 (16.2)		1 (7.6)	2 (15.3)	3 (2.30)	-	1 (7.6)	13 (4.8)	13 (4.23)
1/40- 1/80	-		1 (9.0)	-		6 (54.5)	3 (75.0)		1 (9.0)	1 (25.0)	2 (18.1)	-	1 (9.0)	4 (4.1)	11 (3.58)
1/80- 1/160	-		-	-		-	-		-	1 (50.0)	2 (66.3)	1 (50.0)	1 (33.3)	2 (1.1)	3 (0.97)
1/160	-		-	-		-	-		-	-	1 (100.0)	-	-	-	1 (0.32)
n =	188		89	41		102	27		56	9	41	3	19	268	307

Figures in parentheses indicate percentages

U = Urban

R = Rural

n = Total number of children

Table 5

PRE-IMMUNIZATION BASAL ANTIBODY STATUS IN CHILDREN
FROM URBAN AND RURAL AREAS

Group	Antigen	No. and percentage of children with antibody levels					
		IU/ml < 0.001	> 0.001-<0.01	> 0.01-0.05	> 0.05-<0.1	> 0.1-<0.5	%over 0.01
		Titre < 1/10	1/10-1/20	1/20-1/40	1/40-1/80	1/80-1/160	%over 1/160
Urban n=268	Tetanus	207 (77.2)	20 (7.4)	22 (8.2)	19 (7.0)	-	15.2
	Diphtheria	202 (75.3)	28 (10.4)	21 (7.8)	17 (6.3)	-	14.1
	Pertussis	112 (41.7)	136 (50.7)	18 (6.7)	2 (0.7)	-	0.0
Rural n=307	Tetanus	281 (91.5)	11 (3.5)	8 (2.6)	7 (2.2)	-	4.8
	Diphtheria	249 (81.1)	36 (11.7)	16 (5.2)	6 (1.9)	-	7.1
	Pertussis	84 (27.3)	196 (63.8)	24 (7.8)	2 (0.6)	1 (0.3)	0.4

Figures in parentheses indicate percentages

Table 6

POST-IMMUNIZATION ANTITETANUS ANTIBODY LEVELS IN
CHILDREN FROM TWO DIFFERENT GROUPS

Group	Dose	Total	No. of children with antitetanus antibody levels				Percentage over 0.01 IU/ml
			< 0.001	> 0.001-<0.01	> 0.01-<0.1	> 0.1-<1.0	
Urban	Basal	268	207 (77.2)	20 (7.46)	41 (15.2)	-	14.9
	1st	213	140 (65.7)	21 (9.8)	41 (19.2)	11 (5.1)	24.4
	2nd	125	21 (16.8)	18 (14.4)	74 (59.2)	12 (9.6)	68.8
	3rd	80	2 (2.5)	6 (7.5)	54 (67.5)	18 (22.5)	99.0
Rural	Basal	307	281 (91.5)	11 (3.5)	15 (4.8)	-	4.8
	1st	141	110 (78.0)	10 (7.0)	20 (14.1)	1 (0.70)	14.8
	2nd	80	15 (18.7)	17 (21.2)	43 (37.5)	5 (6.25)	60.0
	3rd	50	3 (6.0)	4 (8.0)	32 (64.0)	11 (22.0)	86.0

Figures in parentheses indicate percentages

Table 7

POST-IMMUNIZATION ANTIDIPHTHERIA ANTIBODY LEVELS
IN CHILDREN FROM TWO DIFFERENT GROUPS

Group	Dose	Total	No. of children with antidiphtheria antibody levels				Percentage over 0.01 IU/ml
			< 0.001	> 0.001-<0.01	> 0.01-<0.1	> 0.01 -< 1.0	
Urban	Basal	268	202 (75.3)	28 (10.4)	37 (13.8)	1 (0.37)	14.1
	1st	213	165 (77.4)	17 (7.9)	38 (17.8)	3 (1.4)	19.2
	2nd	125	7 (5.6)	41 (32.8)	67 (53.6)	10 (8.0)	61.6
	3rd	80	6 (7.5)	10 (12.5)	46 (57.5)	18 (22.5)	80.0
Rural	Basal	307	249 (81.1)	36 (11.7)	22 (7.1)	0	7.1
	1st	141	105 (74.4)	21 (14.8)	14 (9.9)	1 (0.70)	10.6
	2nd	80	13 (16.2)	21 (26.2)	43 (53.7)	3 (3.75)	57.5
	3rd	50	6 (12.0)	6 (12.0)	28 (56.0)	10 (20.0)	76.0

Figures in parentheses indicate percentages

Table 8

POST-IMMUNIZATION ANTIPERTUSSIS ANTIBODY LEVELS
IN CHILDREN FROM TWO GROUPS

Group	Dose	Total	Antipertussis antibody titre							Percentage over 1/160
			< 1/10	1/10	1/20	1/40	1/80	1/160	1/320	
Urban	Basal	268	112 (41.7)	137 (51.1)	13 (4.8)	4 (1.4)	2 (0.74)	-	-	0
	1st	213	63 (29.5)	101 (47.4)	29 (13.6)	18 (8.4)	2 (0.93)	-	-	0
	2nd	125	31 (24.8)	31 (24.8)	33 (26.4)	18 (14.4)	10 (8.0)	2 (1.6)	-	1.6
	3rd	80	11 (13.7)	7 (8.75)	17 (21.2)	11 (13.7)	8 (10.0)	22 (27.5)	4 (5.0)	32.5
Rural	Basal	307	103 (33.5)	176 (57.3)	13 (4.2)	11 (3.5)	3 (0.9)	1 (0.32)	-	0.32
	1st	141	47 (33.3)	81 (57.4)	4 (2.8)	8 (5.6)	0	1 (0.70)	-	0.70
	2nd	80	24 (30.0)	13 (16.2)	23 (28.7)	17 (21.2)	2 (2.5)	1 (1.25)	-	1.25
	3rd	50	14 (28.0)	8 (16.0)	8 (16.0)	3 (6.0)	3 (6.0)	12 (24.0)	2 (4.0)	24.0

Figures in parentheses indicate percentages

BASAL VERSUS POST-IMMUNIZATION ANTITETANUS, ANTIDIPHTHERIA
AND ANTIPERTUSSIS ANTIBODY LEVELS IN CHILDREN FROM DIFFERENT AREAS

Group	Immunization status	Antigen	No. and percentage of children with antibody levels IU/ml				
			> 0.001-<0.01	>0.01-<0.05	>0.05-0.1	>0.1-<0.5	>0.5-<1.0
			1/10 - 1/20	1/20- 1/40	1/40-1/80	1/80-1/160	1/160-1/320
Urban	Basal (n=268)	Tet.	23/(8.5)	32/(11.9)	5/(1.8)	-	-
		Diph.	28/(10.4)	21/(7.8)	17/(6.3)	-	-
		Pert.	136/(50.7)	18/(6.7)	2/(0.7)	-	-
	Post-imm. (n= 80)	Tet.	6/(7.5)	34/(42.5)	20/(25.0)	10/(12.5)	8/(10.0)
		Diph.	10/(12.5)	24/(30.0)	30/(37.5)	7/(8.75)	3/(3.75)
		Pert.	24/(30.0)	11/(13.7)	8/(10.0)	22/(27.5)	4/(5.0)
Rural	Basal (n=307)	Tet.	11/(3.5)	8/(2.6)	7/(2.2)	-	-
		Diph.	36/(11.7)	16/(5.2)	6/(1.9)	-	-
		Pert.	186/(63.8)	24/(7.8)	2/(0.6)	-	-
	Post-imm. (n= 50)	Tet.	4/(8.0)	24/(48.0)	8/(16.0)	6/(12.0)	5/(10.0)
		Diph.	6/(12.0)	20/(40.0)	8/(16.0)	6/(12.0)	4/(8.0)
		Pert.	16/(32.0)	3/(6.0)	3/(6.0)	12/(24.0)	2/(4.0)

n = Total number of children

Figures in parentheses indicate percentage

Table 10
PERCENTAGE SEROCONVERSION IN CHILDREN FROM TWO
GROUPS FOLLOWING DOSES OF TRIPLE ANTIGEN

Stage of Immunization	U/R	Percentage of children in tetanus/diphtheria seroconversion	Value of Z* tetanus/diphtheria	Remarks
One month after	URBAN	24.4/19.2	1.33	NS
1st dose of DPT vaccine	RURAL	14.8/10.6	1.06	NS
One month after	URBAN	68.8/61.6	0.7	NS
2nd dose of DPT vaccine	RURAL	60.0/57.5	0.32	NS
One month after	URBAN	90.0/80.0	1.78	NS
3rd dose of DPT vaccine	RURAL	86.0/76.0	1.28	NS

* Z = Fischer's Z test for percentages,

NS = Not significant

$$Z = \frac{P_1 - P_2}{\sqrt{PQ (1/n_1 + 1/n_2)}}$$

Fischer's
Fischer's

DISCUSSION

Selection of groups :

Since the study populations involved two heterogenous groups of children, stemming from different ecosystems, it is possible to derive conclusions in the context of entire community, in general.

The socioeconomic, nutritional and environmental status differed in both the study groups. However the antigen, its source, dosage and intervals between doses and mode of administration remained identical, so that a comparison of the immunological status of each of these groups could reveal aberrations/alterations in response towards primary childhood immunization with DPT vaccine.

It is apparent from the results that awareness towards the childhood immunization programme is greater in the urban community than the rural, the probable basis of this being, in the case of the latter, illiteracy and lack of knowledge concerning modern immunization practices made available by our Government through its health schemes which emphasize maternal and child welfare programmes. Once again proximity of the immunisation centres along with their paraphernalias also accounts for the substantial coverage of child populations involved during mass immunization campaigns.

The significant difference between the drop-out rates between these two groups becomes apparent when it

is noted that up to 50 % of the urban children completed the time-bound schedule of the three advocated doses of triple antigen, as against only 25 % in the rural community having achieved the same goal.

Another noteworthy observation is the higher age-range of the rural children reporting for primary immunization with DPT vaccine. This may well be due to several of such children having had clinical or subclinical attacks of whooping cough, or diphtheria with resultant naturally-acquired antibodies against these diseases.

Basal or naturally-acquired immunity
against diphtheria, pertussis and tetanus :

Our results have mentioned in detail the preimmunized basal or naturally acquired specific antibodies against diphtheria, pertussis and tetanus, in two groups of children. As many as 15 % of urban children of age range 4-6 months possessed protective levels of antibodies (0.01 IU/ml, Newell et al., 1971; Ypsen, 1946) against tetanus and diphtheria, while only a single child from the rural area had an antibody titre against whooping cough considered as protective (1/320. Miller et al., 1947; Sako, 1947). However 70 % of the rural child population

had detectable level of antibodies against whooping cough. Only 10 % of the children from rural area had protective antibodies against diphtheria or tetanus. However, children in the higher age range i.e. between 9-12 months, in spite of negative history of earlier immunization with DPT antigens have demonstrated higher levels of naturally acquired antibodies against diphtheria or pertussis.

A possible explanation of such findings of naturally acquired antibodies in these heterogenous groups might be the maternal immunization against tetanus, which is customary and is followed, as a routine practice, at deliveries occurring at urban institutions. During the neonatal period these could be passively transferred (maternal antibodies) across the placenta and appear in the infant and child.

Our earlier experience (Gaitonde and Chanderkar, 1972; 1978) also substantiates this hypothesis. The same mechanisms could also account for the larger number of children exhibiting antidiphtheria antibodies, these being, probably, transferred from the mothers in the urban area. As against this background, rural children, as a rule, develop natural antibodies against whooping cough, or diphtheria, as a consequence of natural infections. In other words, children in rural area are at much greater risk of developing neonatal or childhood

tetanus than children in the urban area and, since the half-life of maternally transferred antibodies is not more than 4-6 months, all children in the age-range 6 months and above are at equal risk of suffering from these diseases during the regimen-period of active immunization.

The presence of passively-acquired maternal antibodies is believed, in some cases, exert an inhibitory effect on active antibody formation in the infant, the effect depending upon the quanta and qualities of the vaccination antigens. However, with inocula of high antigenic potency, such as toxoids, active formation of antibodies is barely affected. Thus, antibody formation in the infant against diphtheria, tetanus and perhaps, against the coupled pertussis component of DPT is hardly affected by passively transferred maternal antibodies. Since pertussis infection is most virulent during the second six months of life, the vaccine should be administered as early as 2 months and no later than 7 months following birth; customarily, it is administered between the 4th and 5th months of life.

In vaccination against poliomyelitis and measles, maternal antibodies do inhibit development of active immunity in the infant as the half-life of these maternally acquired antibodies is about 6 months on the

average so that vaccination with measles and poliomyelitis may effectively be administered when the child is about 9 months old.

Considering the preimmunization levels of antibodies against tetanus, diphtheria and pertussis, there was significant difference in the number of children exhibiting detectable as well as protective level of basal antibodies against all these three infections when compared with each other. The number of children possessing antitetanus antibodies being double the number of those possessing antidiphtheria or antipertussis antibodies. Totally there were 15 % of children who showed antitetanus antibodies without the presence of either antidiphtheria, or antipertussis antibodies. These observations were entirely dependant on the earlier encounter with the any of these antigens by child or mother in the preimmunization period.

Post-immunization antibody levels :

Thus, there is a lack of published reports on the immunologically proved efficacy of doses of triple antigen in children in the context of the Indian population. However, the results of some of the earlier trials conducted soon after introduction of the combined

vaccine, have been published and has been as mentioned in introduction, these have already stressed the importance of minimal three-dose temporally spaced schedule for the achievement of maximal efficacy during the most susceptible periods of life.

Considering the results of the present dissertation, it has, herein, been highlighted that, at each dose level, there was a definite increment in the number of children attaining higher titres of antibodies, a single dose of DPT vaccine being entirely inadequate to produce hard immunity, thus failing to achieve protective antibody levels in the majority of children previously immunized.

Tetanus.

Although 15 % of the urban and 5 % of the rural population of children showed protective basal antibody levels, these did not interfere in any manner, with the mechanism of further antibody production following a single dose of DPT vaccine. In fact, the percentage of such children, who showed raised antibody titres, increased and, even following a single injection, nearly 15 % were above the protective limits. With the second dose at an interval of 4-6 weeks, there was a further rise and 60 % of the rural and 68.8 % of the urban

children showed evidence of protective antibody levels. Thus, in mass immunization programmes, even with 50 % of the total drop-outs following 2 doses of DPT, it may be assumed that, in all 25 % population of children can have adequate antibody levels against tetanus.

As per our earlier studies, mentioned above, the antibody titres in the undernourished, rural children were apparently, low in comparison with those of healthy children. However, if the only criterion applied is that of the protective antibody level, there is no significant difference between these two groups of children immunized with DPT.

Thus, to the present author's knowledge, no attempts have been made to reduce the three requisite spaced doses of triple antigen to a single one, while concomitantly ensuring the same level of efficacy in terms of the protective antibody level. Nevertheless, attempts are in progress aiming at the achievement of single dose immunization against tetanus with concentrated toxoid. Our own studies (Gaitonde and Chanderkar, 1978) and those of other investigators (Stanfield et al., 1973) have shown encouraging results in this regard. However, the same methodology has not been applied specifically to triple, or combined antigen, to date. Perhaps the difficulty lies with the pertussis fraction,

which is of low immunogenicity and, in addition, possess the potential for the side-reactions commonly encountered with the administration of DPT antigen, which precludes its further concentration in a vaccine of the combined type.

Nevertheless, a single dose of DPT could be useful in terms of enhancement of the cell-mediated immune response, which has been shown to follow even a single dose of tetanus toxoid, in individuals with normal nutritional status (Gaitonde and Chanderkar, 1978). This calls for further studies on cell-mediated immune responses in children following DPT immunization.

There is definite evidence (Cockburn, 1955) that babies respond exquisitely to both, diphtheria and tetanus toxoids. Only when high levels of transplacentally-acquired antitoxin are present at the time of vaccination, has a diminution in the level of its active production been observed. At any rate, by the age of 4-5 months, the transplacentally-acquired antitoxin will, generally, have fallen to levels which are insignificant in this respect (Barr, 1953; Bover et al., 1953; Di Sant, 1949, 1950; Greenberg and Fleming, 1951; Peterson and Christie, 1951). Our results confirm this in as much as 90 % of the children developed protective antibody levels following 3 doses of the vaccine.

Diphtheria.

Although diphtheria is an antigen whose equivalent potency is almost the same as that of tetanus, its antitoxin protective levels were developed in 60 % of children after 2 doses and 80 % following the completion of the immunization schedule. Although the percentages of children responding to each of the immunising doses were somewhat lower as compared to those in the case of tetanus, there was no significant difference between the responders, if the only criterion applied is that of the protective level. Even in the case of some of the rural children, who possessed basal antibody levels as a consequence of a subclinical infection, the development of enhanced immunity following active immunization was not affected. There is very little evidence that mixing an adsorbed diphtheria toxoid with pertussis vaccine increases the immunizing potency of the toxoid. Certainly, Ordman and Grasset (1948) have demonstrated higher antibody titres with increment in the content of H. pertussis. Bell (1948) found that 10 % of 236 children over 5 months of age were Schick-positive one year following two doses of alum-precipitated toxoid, as compared with 3 % of 271 children administered two doses of adsorbed diphtheria-pertussis antigen. At any rate, it is certain that the potency of the adsorbed diphtheria toxoid is not reduced by the addition of pertussis vaccine (Simon and Craster, 1941).

Pertussis.

Our results show conclusively (Table 8 and 9) that the pertussis fraction of DPT vaccine is, - immunogenically, the weakest of the 3 component antigens. Nevertheless, taking into account the higher percentage of children carrying some level of basal antibodies, the further immunological response was, again, adequate. It has been shown that precipitins against B.pertussis are present in vaccinated persons, or those suffering from whooping cough (Aftendelians and Connor, 1973). This finding has also been reported in the Indian context by Saran et al., (1973). Our results are not inconstistant with these data on naturally acquired antipertussis antibodies.

The post-immunization titres following one to three doses of DPT antigen showed a slow anitbody response in all the children even upon completion of the whole schedule, only few children exhibiting titres over 1/80. There was no evidence of any interference by normally present antipertussis antibodies in the process of generation of the immune response following doses of DPT vaccine. Once again, as in the case of tetanus and diphtheria, there was no significant difference in the responders from the two groups of children studied in the present investigation.

SUMMARY AND CONCLUSIONS

Data on controlled clinical trials with DPT vaccine are still meagre in the Indian context. This is because of the inherent logistic and other difficulties commonly encountered in all economically developing nations, not excluding our own, which have forced us to base our immunization programmes and policies on data provided by the WHO, which as a rule have accrued from studies performed outside the Indian subcontinent. The present investigation was therefore, undertaken, to study actual situation in Bombay City and its immediate environs.

Two groups of children from different ecosystems, urban and rural and differing in their socioeconomic and nutritional status, were studied for immunological responses to one of the most widely utilized of combinations of antigens, DPT vaccine, employing the 3 dose regimen.

The data were analysed in the context of the pre- and post-immunization antibody levels at each of the dose intervals.

As far as the pre-immunization (basal) antibody levels were concerned, there was no evidence to substantiate certain views on the so-called naturally acquired antitetanus, antidiphtheria, nor antipertussis antibodies in children. Although some of the children did exhibit higher antidiphtheria or antipertussis antibody levels

with higher age range, their origin might probably be traceable to subclinic infections more prevalent in rural areas. In the younger age group (3-5 months) in the urban locality, there was evidence of maternally transferred antitetanus antibodies.

The post-immunization antidiphtheria, antipertussis and antitetanus were similar in both the groups, which were from the same age ranges, irrespective of the ecosystem from which the child population stemmed. Thus a single injection of DPT antigen evoked some response in both groups of children with nearly 15 % of them covered under the protective levels for tetanus and diphtheria. Similarly, with the second dose, as many as 60% of the children were beyond the protective range. With completion of the immunization schedule, of three temporally spaced injections, however, 90 % of the children showed adequate antibody levels for protection against the above mentioned two diseases. However the response towards pertussis fraction of the triple antigen was slower as adequate antipertussis antibodies developed in over 30 % of children only after administration of the third and final booster dose.

The children coming from different ecosystems, but belonging to similar age groups responded similarly, to one to three doses of DPT vaccine.

The periodic elevation in the levels of specific antibodies to the administered immunogens did not show any significant difference between the two groups of children.

Combined immunization against diphtheria, pertussis and tetanus with DPT vaccine is one of the commonest prophylactic procedures in general use, and the efficacy of this vaccine still outweighs complication in preventing mortality and morbidity in children from urban and rural areas of an economically developing country such as our own.

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