

A RANDOMIZED CONTROLLED FIELD TRIAL OF THE EFFICACY OF A SIMPLIFIED
MATERNAL-IMMUNIZATION SCHEDULE IN REDUCING NEONATAL
TETANUS DEATHS IN RURAL SIERRA LEONE

by



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A Thesis

Submitted to the School of Graduate Studies
in Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

September 1979

REDUCING NEONATAL TETANUS DEATHS BY MATERNAL IMMUNIZATION

MASTER OF SCIENCE (1979)
(Design, Measurement and Evaluation:
Health Care Research Methods)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: A Randomized Controlled Field Trial of the Efficacy of a
Simplified Maternal Immunization Schedule in Reducing
Neonatal Tetanus Deaths in Rural Sierra Leone

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NUMBER OF PAGES: ix, 98

ABSTRACT

The purpose of this thesis is to describe a research strategy for evaluating the efficacy of a reduced dose maternal tetanus immunization schedule in reducing neonatal tetanus deaths in Sierra Leone.

Neonatal tetanus has been demonstrated to be a major cause in infant death in areas where sanitary midwifery is unavailable. A review of the literature reveals evidence for reduction in neonatal tetanus deaths by maternal immunization using a conventional three dose adsorbed tetanus toxoid.

In Sierra Leone, as in many developing countries, the resources are unavailable to maintain a three-dose schedule in the relatively inaccessible interior of the country. A two-dose schedule of a concentrated adsorbed toxoid has been recommended as an alternative by the World Health Organization. Research on simplified immunization schedules has been reviewed; the results are inconclusive.

A double-blind randomized placebo-controlled design has been selected to address the research questions. Various aspects of this design are discussed with respect to methodologic and ethical issues.

Procedures for carrying out the trial and for the assessment of neonatal tetanus death in a remote chiefdom in Sierra Leone are proposed. A procedure for the analysis of outcome data is presented.

ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation for the substantial contributions made by others to the development of this thesis.

The thesis supervisor, Peter Tugwell, and the readers Mike Gent and Jack Sibley, offered much guidance and encouragement, and even in pressing circumstances remained tolerant and open to the author's needs.

Eme Essien, George Gage and Mark Magenheim provided innumerable bits of information about West Africa and Sierra Leone.

Students and staff in the department were regularly called upon for methodologic or statistical advice and opinions.

Susan Hanna contributed many lunch hours and evenings complying without complaint to the unreasonable last minute requests for typing and organization of the manuscript.

Special thanks to M.J., who by managing, entirely on her own, all the preparations required for 18 months abroad, allowed this thesis to be written in the available time.

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CHAPTER 1

INTRODUCTION

1.1 Health Objectives in Sierra Leone

In the National Development Plan 1974/75 to 1978/79 the Sierra Leone government has stated its objectives in improving the state of health of its citizens. Emphasis has been placed on disease prevention rather than cure, on maternal and child health, and on a rural health infrastructure. The highest priorities have been placed on the control of tetanus, measles, tuberculosis, leprosy, and whooping cough, both because of high incidences of these diseases and because of the possibility for reduction in morbidity and mortality through immunization.

1.2 Tetanus

1.2.1 Magnitude of the Problem of Tetanus

Tetanus is a disappearing disease in many developed countries, probably due to improvements in living conditions, easier access to medical care, and the mechanization of agriculture, but particularly to the introduction of large-scale active immunization programmes. Tetanus remains a serious problem in developing countries; incidences

in some tropical countries appear to be increasing (7).

This, however, probably represents an improvement in the reporting system rather than an increase in disease. In Sierra Leone, tetanus is listed among the ten principal causes of death (7).

1.2.2 Magnitude of the Problem of Neonatal Tetanus

Tetanus of the newborn is most likely to occur where births are attended by midwives who are unaware of the need for sanitary handling of the umbilicus. Tetanus bacilli are found all over the world but seem to be in higher concentrations in hot, wet regions with soils rich in organic matter. The remote tropical areas, which are then suggested to be the areas of highest incidence, are also the areas with very limited reporting systems. Estimates have been made in various defined areas, particularly those in which specific programmes for the reduction of tetanus mortality have been introduced. In an area of Columbia, Newell (43) found an incidence of 110 deaths per 1000 live births; Schofield et al (52), 60-80 per 1000 in New Guinea; and Berggren (4) in Haiti describes an isolated valley where the rate before intervention was 270 per 1000 live births.

In Sierra Leone, two hospital admissions surveys, one in Freetown (8) and another in Segbwema (61) found tetanus to be the second greatest cause of death in children. A survey done in the Serabu area of Sierra Leone showed tetanus to be the leading cause of death in neonates, with an incidence of approximately 115 per

1000 live births.

Treated case-fatality estimates range from 48 to 73% (60). However, onset of the disease is very rapid and prognosis is related to the length of the incubation period. It is likely, then, that those infants surviving the length of time that is required to gain access to medical care are the least severe cases. Newell (43) in Columbia has estimated the untreated case fatality rate at 96%.

1.3 Natural History of Neonatal Tetanus

1.3.1 An Illustrative Example

The tetanus bacillus is found in the superficial layers of soil and in the intestinal tracts of humans and certain animals, particularly horses, cows, goats, and poultry. It is most frequently encountered in regions with hot damp climates, in soil rich in organic matter. Neonatal tetanus is usually caused by the infection of the umbilical stump.

With the above as background, the following excerpt may serve to illustrate the conditions which permit tetanus infections. The excerpt is from Wilkinson (60), describing the traditions near Segbwema in the Eastern Province of Sierra Leone.

"The people comprise a predominantly agricultural community ... Most of the patients come from villages of fewer than 500 people. There are no horses and few cows in this area, but it is common to see goats, hens, sheep, and sometimes pigs wandering around the houses. At night the hens are taken indoors,

and other animals often shelter on the veranda ...

There are relatively few trained village midwives, and, so far as is known, all the cases here discussed were delivered by traditional village midwives -- usually Bundu Society elders. Birth usually takes place on a grass mat spread on the earth floor of a mud-walled thatched house. According to the custom of the local Mende tribe, as soon as the baby is born a woman will run to the village and borrow a particular knife kept by an old man; the knife may be hanging in a raffia bag on the wall, on the top of a cupboard, or in a box together with other possessions. The umbilical cord is tied with native spun thread, then "medicine" is applied by the midwife, and subsequently each morning by the grandmother. The application usually consists of the juice from a young banana shoot, squeezed out by rolling it between the hands. Sometimes leaf fragments are added. It is interesting to note that the banana trees are commonly grown behind the native houses; it is customary to throw house sweepings and refuse around the bottom of the trees, and animals root among this in search of food scraps. A young banana shoot originates from its parent stem below ground and thus comes into close contact with the debris around the tree base."

1.3.2 Natural History

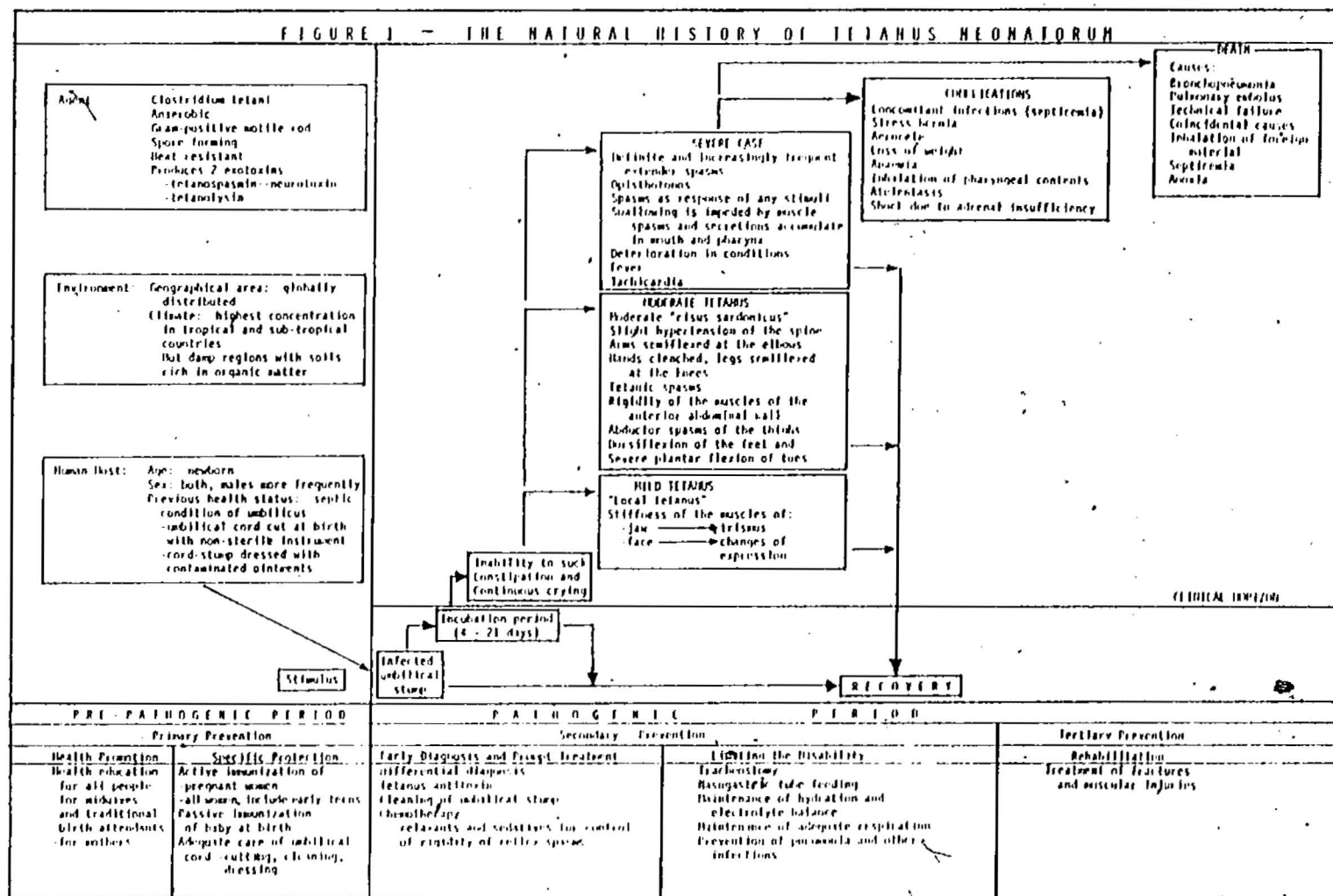
Details of the natural history of neonatal tetanus are shown in Figure 1. The general format for this style of natural history presentation is explained in Appendix A.

1.4 Conclusions

The high incidence and high case-fatality rate of neonatal tetanus in Sierra Leone, and the possibilities for its prevention, have given the control of this disease a high priority. In the

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FIGURE 1 - THE NATURAL HISTORY OF TETANUS NEONATORUM



succeeding chapters, previous research in the prevention of neonatal tetanus will be discussed and a protocol for the teaching of a simplified immunization programme for the reduction in incidence of the disease will be presented.

CHAPTER 2

LITERATURE REVIEW

2.1 Historical Perspective

2.1.1 Maternal Immunization and Neonatal Protection

In 1923, Broeck and Bauer (6) reporting on their studies in Peking, demonstrated a strong relationship between the presence of tetanus bacilli in the stools of mothers and the presence of antitoxin in blood obtained from the umbilical cord at birth, and concluded that the antitoxin is capable of crossing the placenta. In the same year, the French Veterinarian Ramon (46) reported his discovery of a tetanus toxoid. Prior to this time, tetanus antitoxin had been commonly used for the passive immunization of draft animals; but now, active immunization was possible.

In 1927, Nathan-Larrier et al (41), on the basis of these two studies, hypothesized that the active immunization of a pregnant woman may cause sufficient antitoxin to cross the placenta to passively immunize the neonate.

2.1.2 Testing the Nathan-Larrier Hypothesis

This hypothesis remained untested for 30 years. In 1950, the World Health Organization (63) recommended that the hypothesis be examined for the potential of reducing neonatal tetanus deaths. The first study was started in

1959 in New Guinea and reported in 1961 by Schofield et al (52). Despite methodologic problems (discussed later), promising results were obtained. Subsequently, a randomized controlled trial was done in Columbia by Newell (42,43,44). MacLennan and his group (1,28,29,35,45) followed Schofield with other studies in New Guinea. These, and the before-after incidence studies in Haiti (3,4,38), are the only studies which relate maternal tetanus immunization to reduction in neonatal tetanus deaths. Since that time several studies have been reported associating various immunization schedules with increased serum antitoxin levels.

2.2 Maternal Immunization and Neonatal Tetanus Death: Review of Evidence

2.2.1 Schofield - New Guinea

Schofield, Tucker and Westbrook (52) initiated an analytic survey wherein they intended to immunize pregnant women with three doses of fluid toxoid at 6 week intervals. All women who presented in pregnancy at a Maternal and Child Welfare Clinic were eligible for entry in the study. The proportion of women actually entered into the study and the proportion and reasons for exclusion were unstated. Infants were excluded if the birth was assisted by health care personnel (twelve cases) or if antibiotics were administered for a non-tetanus related problem (nine cases), or if the infant died without being observed (four cases); total live births were 594.

Groups were formed after the immunization phase by dividing the women by the number of injections received. The "control group" consisted of women who had received 0 or 1 injection, either because of a shortage of toxoid or because they presented too late in pregnancy to receive more injections. The "two-dose group" consisted of women seen too late in pregnancy to receive the full course of three injections. (The number of women who could have received three doses but did not, was not specified.) The three-dose group consisted of those women who received all three injections. The proportions of infants in each group were 0-1 dose, 28%; 2-dose, 41%; 3-dose, 31%. Tetanus death assessment was by direct observation of the deaths by unblinded study personnel. The neonatal tetanus death rates were, respectively, 10.0%, 3.42%, 0.57%. The one tetanus death in the three-dose group occurred within a week of the third injection. The authors conclude that three doses of fluid toxoid during pregnancy is fully protective from neonatal tetanus, two doses is two-thirds protective and one dose yields no protection.

The following alternative could, however, explain at least some portion of their results: women who present early in pregnancy are more concerned about health and are thus less likely to incur tetanus infection. The observation that the one-dose group has a higher rate of tetanus death than the initial untreated rate in the area does seem to suggest that this explanation is possible. This unfortunate confounding, plus the potential for bias in unblinded

outcome assessment, and the poorly specified sample and group selection reduces the confidence in the conclusion drawn by the investigators.

2.2.2 Newell - Columbia

Newell (42,43,44) wished to test the hypothesis that three doses of adsorbed toxoid in women (not only pregnant women) would reduce neonatal tetanus mortality. The trial was carried out in a geographically isolated area of Columbia. There were no medical services available within the area, and travel to other areas was restricted by three unbridged rivers. Women were randomly assigned to receive either three tetanus toxoid injections or three influenza injections. Neonatal deaths were assessed in some cases by direct observation of the infected infant by study personnel, but primarily by interview with the mother subsequent to the infant death. The study is reported as a double-blind randomized trial.

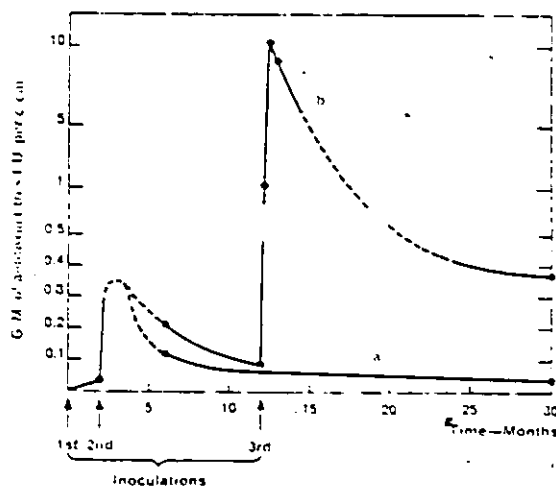
The neonatal tetanus death rates (ranging from 4.0 to 7.9%) were statistically similar among the placebo group, the non-volunteers and the members of the toxoid group who received only one injection. In women receiving two or three doses of toxoid, no neonatal tetanus deaths were observed. The authors concluded that two or three injections of adsorbed toxoid protect against neonatal tetanus for at least four years. This conclusion has been widely accepted.

This trial is open to the following criticisms:

- (i) Less than 60% of the women registered during the survey of the district volunteered to receive any injections. Less than 30% received the scheduled three injections. The high percentage of self-selected exclusions limits the generalizability of the conclusions of the study.
- (ii) Although the contents of the injections were unknown to both subjects and study personnel, the group assignments were known to both. That is, it was known who were assigned to each of groups A and B, but which of A or B was toxoid was unknown. This caused two problems:
 - a) A greater number of refusals among volunteers occurred during the injection procedure, as women in the queue found that one of the preparations was more painful than the other and knew which preparation they were assigned to receive. The resultant groups are thus less comparable than those that would have been formed if the injections had been indistinguishable.
 - b) The knowledge of group assignment could have resulted in the unblinding of the study personnel. The outcome assessment personnel would soon suspect which group had been injected with toxoid as a differential death rate started to appear between groups. The possibility of biased assessment, then, cannot be eliminated.

- (iii) The authors state that the results of the subgroups receiving two doses and three doses within each treatment group were undifferentiable and so, the data from the subgroups were combined. The birth rates and death rates cannot be obtained for each of these subgroups separately. The two-dose subgroup is only about one-third the size of the three-dose subgroup; it may well be that the difference in rates was statistically undifferentiable because of insufficient sample size rather than a true similarity in rates.
- (iv) The conclusion drawn from the results of the two-dose subgroup may overstate the length of time for which two doses are "protective". As in Schofield's study, the one and two-dose subgroups were formed in large part by delivery occurring before the administration of the next scheduled injection. The two-dose subgroup is, then, primarily a group of pregnant women. If one examines the rise in antitoxin levels associated with injection of toxoid, (see Figure 2), it can be seen that a much higher antitoxin level exists shortly after the second injection than three or four months later. Since it is known that the two-dose group did not receive a third dose, scheduled six weeks after the second, it is likely that at least some portion of this group would have given birth during the antitoxin peak following the second injection.

Figure 2



Tetanus antitoxin titers in the blood of persons receiving (a) two doses and (b) a third dose, of fluid tetanus toxoid (from EVANS (23)).

- (v) The same point may be made about antitoxin levels following the third dose, but it is likely that a much smaller proportion of the three-dose subgroup were pregnant during the immunization phase.

2.2.3 MacLennan - New Guinea

MacLennan and his associates have done several studies following Schofield's original work in New Guinea. These studies are primarily antitoxin level comparisons of different toxoids and

schedules, but two points can be noted from the 1965 study (35).

- (i) A five-year follow-up of 900 of the women who received all three shots of fluid toxoid in Schofield's study revealed no neonatal tetanus deaths in 1500 births.
- (ii) Although the women in the antitoxin studies (1,28,29, 35,45) were followed for the occurrence of neonatal deaths, the sample size is insufficient to make conclusions about the efficacy of death reduction of the five different schedules tested. The total number of births was 120, the largest number in a group was 33; pre-intervention tetanus death rate, 60-80 per 1000 births.

2.2.4 Haiti

The tetanus control programmes attempted in a defined region in Haiti have been discussed by Marshall (38), Berggren (4) and Berggren and Berggren (3). Incidence figures were obtained from the regular census and from hospital records. The incidence of neonatal tetanus death decreased from 110 per 1000 to 50 per 1000 live births with an immunization programme for pregnant women. This rate reduced to 9 per 1000 when a mass campaign was completed which immunized at least 72% of all women with three injections of adsorbed toxoid. In the five years following the immunization of the first 60,000 women, only one case of neonatal tetanus has been found where the mother received all three injections. While some of the reduction in death is probably due to increased awareness of the public to

tetanus, and thus to improved midwifery practices, the results do lend weight to the previous studies.

2.2.5 Conclusions

While the evidence for three-dose efficacy is not without methodologic problems, it is difficult to suggest an alternative explanation that could account for the results of the various studies discussed. The same cannot be said of the two-dose evidence. With respect to neonatal tetanus death outcome, there is insufficient evidence to conclude that two doses provide sufficient protection for more than a short time following the second injection.

2.3 Serum Antitoxin Studies

2.3.1 Methods of Determining Serum Antitoxin Level

An outline of the two techniques for antitoxin level determination is presented in Appendix B. In many of the studies in the tetanus literature, the accuracy of the neutralization test has been traded for the relative cheapness of the haemagglutination test. However, based on the results of several comparisons of the two methods (e.g. Edsall (22), Hardegree (29), Rey (50)), the following conclusion was drawn at the 1978 meeting in Guadeloupe of the International Association for Biological Standardization (37):

"Although a broad correlation exists between tetanus antitoxin titres as measured in vitro by haemagglutination titers, and in vivo by the mouse neutralization method, it was concluded that the HA test was not sufficiently reliable for either the determination of future prophylaxis of individuals, or for evaluating the efficiency of single dose schedules, but it might be used with caution and a certain amount of difficulty to assess the proportion of susceptible individuals in a population."

This strong statement throws into doubt the results and conclusions of the many immunization studies which use HA levels as the only measure of outcome.

2.3.2 The "Protective" Antitoxin Level

Several studies have been done which use the "protective" serum antitoxin level as the outcome measurement for tetanus immunization trials. This level, 0.01 IU/ml, was originally determined as the level at which a guinea pig would survive a very large tetanus toxin challenge (53). The studies usually refer (often indirectly) to one of three sources as the basis for the selection of this level. Of these three, McComb (39) is a review of primarily animal studies wherein the basic evidence cited is the lack of contradictory evidence. Wolters and Dehmel (62) immunized themselves with toxoid, determined their antitoxin levels as .007 and .01 IU/ml, then injected "fatal" doses of toxin. They lived to publish the results. The one study (1,28,29,35,45) which specifically addresses the question of

protection of neonates with maternal antitoxin levels concludes that 0.01 IU/ml does protect the neonate. This was an uncontrolled study in which 120 births were observed in 134 immunized mothers without any tetanus deaths. The neonatal tetanus death rate in the area was 60-80 per 1000 live births. In the data presented, 12% of the women's sera did not reach the 0.01 IU/ml level. It is suggested that this data offers little justification for the selection of 0.01 IU/ml as the protective level.

2.3.3 Evidence from Antitoxin Level Studies

The limitations of antitoxin level studies restrict their use for determining the efficacy of a tetanus immunization schedule in reducing neonatal tetanus death. However, use may be made of some of the results for hypothesis-generating and for details necessary for the design and implementation of an efficacy trial.

Several studies in animals (1,29,51) and in humans (28,29,35,56) have shown that the distribution of titres following adsorbed toxoid immunization is higher and longer lasting than the distribution of titres following fluid toxoid immunization. The relationship of high and low toxoid concentrations with high and low adjuvant concentrations has been examined in humans (36) and in animals (47). In both studies, the high dose - high adjuvant toxoid produced higher antitoxin levels than the low dose - low adjuvant combination. After

a year, the difference was more pronounced. The low dose - low adjuvant toxoid is a commonly used toxoid (e.g. Canada, Haiti, India) for human immunization.

In a comparison of two doses of high dose - high adjuvant toxoid with three doses of a conventional adsorbed toxoid (32), the antitoxin levels after one month were similar; after twelve months the concentrated toxoid maintained a higher antitoxin level. This study and others (19,54) noted fewer side effects with an aluminum phosphate adjuvant than with other adjuvants.

Dhillon and Menon (15) investigated antitoxin levels at several intervals between the two injections of a concentrated toxoid. Best results were obtained with intervals of at least twelve weeks, although MacLennan (35) found that with a six week interval, the antitoxin levels were already higher than with three doses of fluid toxoid.

Several studies have investigated the possibility of one-injection immunization. MacLennan's group (1,28,29,35,45) found very high antitoxin levels following one dose of an oil adjuvant toxoid; however, medical attention was required for the many abscesses that occurred. Other very high dose toxoids have been used and have shown promise in obtaining high antitoxin titres in adults and children (21,22,54,55,49,59) Stanfield and Gall (54,55) conclude however, that the transfer of antitoxin across the placenta is "more effective and more rapid after a two-dose schedule than after a single dose"; a substantial proportion of the one-dose cord blood samples in their study showed no measurable antitoxin. Edsall's (21,22) promising one-

dose results occurred in teenage girls and young women volunteers. When he repeated the schedules in a "...rural, marginally nourished, multiracial population ... a very large segment of this population (about one-third) scarcely responded at all to this type of one-dose stimulus even with the use of a high-potency toxoid".

2.4 Conclusions

In remote areas, until the problem with the side-effects of the very highly concentrated toxoids have been resolved, the probably marginal efficacy of a one-dose immunization programme with such toxoids may be less preferable than a potentially more efficacious (and more expensive) two-dose schedule. Two-dose concentrated schedules have been shown to increase antitoxin titres in humans to similar levels as three-dose conventional adsorbed toxoids. The concentrated toxoid to be proposed for use in this trial has been shown to produce (with fewer side-effects) antitoxin levels as high as other similarly concentrated toxoids (1936). It is suggested then, that the efficacy of this two-dose maternal immunization schedule be examined in relation to reduced neonatal tetanus deaths.

CHAPTER 3
THE RESEARCH PROPOSAL

3.1 Justification of the Trial

3.1.1 Efficacy of the Standard Immunization Schedule

In the previous chapters, the magnitude of the problem of neonatal tetanus and the means for reducing incidence have been discussed. The weight of evidence for the efficacy of maternal immunization using the conventional three-dose schedule of tetanus toxoid in preventing neonatal tetanus should have precluded any further need for large-scale trials of toxoids or alternative schedules.

3.1.2 Problems with the Standard Immunization Schedule

- (i) In many developing countries, the logistics of mounting at least three separate immunization expeditions throughout the regions of poor accessibility are beyond the means of the country. In parts of Sierra Leone, various reduced dose schedules have been administered unsystematically, without evidence of efficacy, simply for want of the additional toxoids, manpower, and equipment necessary to maintain the three-dose schedule

in rural areas.

- (ii) An additional problem with multiple-dose schedules is that of compliance. The rural people of Sierra Leone have confidence in injections, and will go to considerable lengths to obtain injections. However, the subsistence occupations of most of the people puts heavy demands on their time, so that the more injections required to complete an immunization schedule, the less likely it is to be completed. Completion rates could be improved with increased convenience and accessibility, but the additional expense may be uneconomic compared with alternative programmes or immunization schedules.

3.1.3 Alternative to Maternal Immunization

There are two alternatives to maternal immunization that could be investigated for use in reducing neonatal tetanus.

- (i) The first alternative is the passive immunization of the infant in the first two days of life. It is, at present, not possible to maintain a supply of tetanus antitoxin and personnel to administer the injections in the number of locations at the time required, that would be needed to effect a significant change in incidence. This plan cannot be considered an alternative

to maternal immunization in rural Sierra Leone.

- (ii) The second possibility to reduce incidence would be a strategy to improve sanitation and midwifery practices during and immediately following delivery. The Ministry of Health has initiated a programme to educate traditional birth attendants (TBA's or "grannies") and to supply them with "midwife kits". This, however, is proceeding slowly due to very limited enrollment. This programme is more expensive than the immunization programme, although it will eventually serve more purposes than the reduction in incidence of neonatal tetanus. Unfortunately, it is unknown whether this programme is having any effect; no one has followed up on the "grannies" to ascertain whether the techniques and kits are being used in the prescribed fashion. Furthermore, it is unknown whether the sterile practices, if used, are being undone by the family who may wish to ensure that the appropriate traditional rites are performed. It will take many years to fully implement this programme and to gain acceptance of a modified birth ritual. It would seem, then, given the acceptance of injections, that a simplified maternal immunization scheme would hold more promise for immediate benefit.

3.1.4 Summary and Conclusions

The need for an efficacious alternative programme for the reduction of neonatal tetanus death in Sierra Leone has been established. Maternal immunization, the most promising of the three approaches for the immediate reduction of incidence of the disease has been discussed and a proposed alternate immunization schedule will be described in the next section and in later chapters. This schedule has been recommended to the Sierra Leone Government by the World Health Organization (WHO), but no evidence has been presented that the efficacy of this schedule has been established. The resultant problem is stated clearly by Sir Austin Bradford Hill (30):

"It is certainly not always recognized that it may be unethical to introduce into general use a drug that has been poorly or inadequately tested. The ethical question is, indeed, not solely one of human experimentation, it can also be one of refraining from human experimentation."

3.2 Justification of the Simplified Schedule

On the recommendation of the World Health Organization, the Ministry of Health in Sierra Leone has already changed its immunization plan from the unaffordable three-dose schedule to a schedule of two injections of a concentrated adsorbed tetanus toxoid.

The present lack of availability of such a toxoid in Sierra Leone has prevented the implementation of this plan.

The WHO recommendation would suggest that the efficacy of the two-dose schedule is already established, but as discussed in Chapter 2, there are methodologic problems in the studies from which the evidence was drawn.

However, the aggregation of these studies does suggest promise of benefit with the two-dose schedule. In particular, the high and sustained serum antitoxin levels reported with two doses of the most recent concentrated adsorbed toxoids (19,48) in trials in school children and adults in Senegal would suggest a trial of these toxoids in reducing neonatal tetanus by maternal immunization.

The decision of the Ministry of Health of Sierra Leone to introduce a two-dose concentrated adsorbed toxoid schedule, together with the very limited availability of these toxoids have presented the opportunity for a rigorous and controlled investigation of the efficacy of the two-dose schedule.

3.3 The Research Objectives

3.3.1 The Primary Question

Is the death rate from neonatal tetanus of infants born to mothers who receive the full course of injections of the two-dose concentrated toxoid schedule within two percent of the death rate

from neonatal tetanus of infants born to mothers who receive the full course of injections of the three-dose conventional toxoid schedule?

3.3.2 The Secondary Questions

- (i) Is the death rate from neonatal tetanus of infants born to mothers who receive the full course of injections of the two-dose concentrated toxoid schedule less than one-half of the death rate from neonatal tetanus of infants born to mothers who receive the full course of injections of a placebo schedule?
- (ii) Is the mean of the serum antitoxin levels of women who receive the full course of injections of the two-dose concentrated toxoid schedule less than twice the pooled standard error from the mean of the serum antitoxin levels of women who receive the full course of injections of the three-dose schedule?

NOTE: The following two questions will be addressed conditionally upon:

- (1) the data from more than 80% of all eligible women being obtained.
- (2) a positive response to the corresponding question, 3.3.1 or 3.3.2 (i).

- (iii) Is the death rate from neonatal tetanus in infants born to all women allocated to the two-dose group within two percent of the death rate from neonatal tetanus in infants born to all women allocated to the three-dose group?
- (iv) Is the death rate from neonatal tetanus in infants born to all women allocated to the two-dose group less than one-half of the death rate from neonatal tetanus of infants born to all women allocated to the placebo group?

3.4 Justification of the Design

3.4.1 Description of the Design

The design selected for this study is a double-blind three-group randomized controlled trial. The experimental units will be randomly allocated to one of three immunization schedules. These three immunization schedules include the three-dose tetanus schedule, the two-dose tetanus schedule and a placebo schedule. The appropriate schedule will be offered to all eligible members of each group. The schedules will be disguised such that the members of each group will be unaware of which schedule they have received. All groups will then be followed for the occurrence of end-points. The end-point rates in each group can be compared statistically to determine the probability of the observed difference in rates occurring by chance.

3.4.2 Aspects of the Selected Design

The reasons for selecting this particular design are discussed below in terms of various design aspects.

(i) Why Concurrent Control?

The simplified schedule could be applied to a single group, and followed for the occurrence of end-points. The observed results could then be compared with either the "before" scores, or with a prespecified goal rate. Such a trial would not take into account any immunization inhibiting factors that may exist in the group, nor would any measure of the effect of concurrent events which may effect the neonatal tetanus death rate be available. If these co-interventions cannot be eliminated, one cannot conclude that a change in death rate is due solely to the effect of immunization. This difficulty can be overcome by selecting a concurrent control group which only differs from the immunization group by the experimental intervention.

(ii) Why a Placebo Control?

The third group discussed above is receiving a schedule of injections which will not immunologically protect the neonate from tetanus infection; this is by definition, then, a placebo group. It has been argued that it is unethical to offer a placebo in human

experimentation when a known efficacious alternative exists. The counter-argument is that, in a situation such as exists in rural Nongowa Chiefdom, the efficacious alternative is not, and will not be, available; the trial is thus not causing anything to be withheld from the trial participants.

A placebo group permits the measurement of the degree of effect of otherwise uncontrolled co-interventions. A sufficient number of changes are occurring in rural Sierra Leone, such as TBA education, improved roads, communications and access to medical care, and in traditional birth practices, that make it desirable to monitor the potential effect of these or other co-interventions.

There are two main reasons for wanting to obtain concurrent non-immunized rates. If the two-dose schedule is inefficacious relative to the three-dose schedule, it is still of importance to know whether it is efficacious relative to the untreated rate since the three-dose schedule is presently unaffordable. Secondly, if the observed rates in both schedules differ considerably from the hypothesized rates, one would be concerned about recommending the use of either schedule without evidence that both were efficacious relative to the rate in unimmunized mothers.

In this trial, it is essential to be able to compare schedules within compliant groups. Since compliance is often related to outcome, ignoring, in the placebo group, the self-selective factors that cause people to be compliers could bias the results. For the purposes of maintaining comparable groups, then, the possibility of a no-treatment group has been excluded; the placebo group will be offered a schedule of three placebo injections.

(iii) Why Randomize?

Sir R.A. Fisher, credited with the development of randomization, states in The Design of Experiments (25):

"Apart from the avoidable error of the experimenter himself introducing with his test treatments, or subsequently, other differences in treatment, the effects of which the experiment is not intended to study, it may be said that the simple precaution of randomisation will suffice to guarantee the validity of the test of significance, by which the result of the experiment is to be judged."

There are two classes of alternatives to randomized allocation:

- a) defined systematic allocation
- b) subjective allocation.

Cox (14) summarizes the work initiated by Greenberg (1951) on systematic allocation and by Student (1931) on subjective allocation and concludes, concerning systematic arrangements:

- "a) the arrangement of treatments may combine with a pattern in the uncontrolled variation to produce a systematic error in the estimated treatment effect, persisting over a long experiment or even over a series of experiments. We may begin by thinking this possibility sufficiently unlikely to be disregarded, but this is a matter of personal judgement which cannot be put on an objective basis;
- b) there is likely, even in the most favourable of cases, to be difficulty connected with the estimation of error from such designs."

and concerning subjective allocation:

"...an experiment is in danger of being very seriously affected if the personal judgement of people taking part is allowed to determine the allocation of treatments to units. There is abundant evidence that observer biases occur even in apparently unlikely circumstances, and moreover, even if the arrangement chosen is in fact satisfactory, there is always the suspicion that it may not be, and this will detract considerably from the cogency of the experiment if surprising conclusions are found."

A further benefit of using randomized allocation of treatment is that it facilitates the concealment of the group assignment from the outcome assessment personnel; another arrangement may be identified, possibly incorrectly, by outcome assessors and a systematic bias result.

(iv) Why Double-blind?*

The blinding of assessors should prevent or reduce the occurrence of differential effort being used to seek information from the three groups. For example,

* Throughout this thesis, the terms "blind", "blinded", or "blindness" refer to the withholding of group assignment information from the study subjects or assessment personnel. The term "double-blind" refers to the blindness of both subjects and assessors.

if it is known that the standard three-dose schedule was one of the groups, it may be believed that it is unnecessary to thoroughly investigate the infant deaths in those villages because of knowledge of the efficacy of this schedule. A more concerted effort in the other groups' villages may turn up more neonatal tetanus deaths than in the three-dose villages because of the additional effort alone, not because of a true difference in rates.

The assessor's perceptions may also influence the reporting of data, either intentionally or unintentionally. If the assessor is aware of the senior study personnel biases and expectations, the assessor may tend to report data that conform to those perceived expectations.

A similar bias occurs when the respondent perceives the expectations of the assessor, via the style of questioning or otherwise, and responds in a fashion which concurs with the perceived expectations. Given that the outcome assessment for this trial must be done in an interview situation, it is necessary to blind both assessors and trial subjects. Maintaining the blindness of the personnel responsible for administering the injections reduces the possibility of these personnel modifying dosages or injections, being less thorough

with some groups, or, on the basis of their personal beliefs, adding unintended interventions differentially to groups.

3.5 Selection of the Placebo

3.5.1 Introduction

A number of other vaccines have been considered for use in the placebo group. It may be thought desirable to attempt a placebo which does offer some benefit to this group, which would still be a "placebo" providing it does not alter the incidence of tetanus mortality.

3.5.2 Issues Affecting Selection of Placebo

The following considerations were examined before selecting the preparation to be used for placebo:

- (i) Any benefit offered by the placebo must also be offered the active toxoid groups so that the difference between groups is only the presence of active tetanus toxoid. If this was not done some loss of comparability would occur if for example, the placebo changed rates of birth, or rates of morbidity or mortality of the mothers. A difficulty arises, however, in offering two vaccines simultaneously to the toxoid groups. Some vaccines

interact antagonistically, which may reduce the number of women truly immunized with either vaccine.

- (ii) The placebo must resemble the active toxoid in terms of mode of administration and side effects. The tetanus toxoid will be administered intramuscularly in three injections; it is known to have very few side effects other than local tenderness following injection.
- (iii) If the placebo injection has any true side effects, it must also have real benefit to those to whom it is offered.
- (iv) The placebo must be safe to offer to pregnant women.

3.5.3. Non-tetanus Vaccines as Placebo

These criteria have eliminated the possibility of using other vaccines for the placebo. For example, influenza vaccine (used in Newell's trial) has little benefit in Sierra Leone, is not recommended in pregnant women, and may interact with tetanus toxoid. Cholera injections are painful, and provide very little protection against the disease. BCG is usually administered by scarification and the side effects may require medical attention, and, of course, the visible scar would unblind outcome assessors.

3.5.4 Conclusions

It has been decided, after consideration of the above criteria, that the only placebo which provides comparability between groups without undue hazard is an inert sterile solution (0.9% solution of sodium chloride).

As Hill (30) states, "Although experience shows that the 'dummy' treatment can produce 'side effects', it is at least unlikely that it can produce irreversible harm!"

3.6 Justification of the Outcome Measures

The outcome measures for this trial are neonatal tetanus death and serum antitoxin level.

3.6.1 Neonatal Tetanus Death

It may be argued that, if immunologic tests such as that for serum antitoxin level exist, there is no need to follow the immunized groups for the outcome of death. Groups similar in their capacity to respond to tests made immediately before and shortly after vaccination could be compared for response to toxoids or schedules. The assessment could be made much more quickly with a smaller number of subjects than trials based on the incidence of the disease. The advantages of a reliable immunologic test are such that trials should be based on such tests if they are available.

However, the substitution of test results for actual desired outcome must be based on the "true" relationship between test and outcome. The presently accepted "protective level" of neutralization titres in maternal serum has not been derived from a trial to examine this relationship, but rather, on the "common knowledge" that neonatal tetanus deaths have not occurred in trials where the maternal serum was higher than this level. Many sera in these same trials were below this level without adverse results; it may be that the true protective level is below the presently accepted one. So, to ensure that a schedule which may be efficacious in reducing deaths will not be discarded because of its failure to reach a poorly founded standard, or, conversely, to ensure that a schedule which **increases antitoxin levels** without reducing deaths is discarded, this trial will follow births for the outcome of tetanus death.

3.6.2 Serum Antitoxin Levels

This trial will contribute information towards establishing the relationship between the predictive value of the serum antitoxin levels achieved in this study and the "hard" outcome of neonatal death from tetanus in the Sierra Leone population. If the simplified schedule is less efficacious in producing antitoxin levels, or if compliance problems are of such a degree that a wide distribution of antitoxin levels occur, it may be possible to examine the

relationship between serum levels and death to obtain more information about the "protective" level. Furthermore, additional information such as the achieved blood levels would provide a useful basis for comparison if one of the schedules is selected for use in other areas of the country, since generalizability from efficacy trials is often very limited.

3.6.3 Selection of Neutralization Titre as Serum Level Outcome

The conclusion of the International Association for Biological Standardization in 1978 (see Section 2.3.1) precludes the use of the HA technique in situations where the antitoxin levels of individuals are required. It has thus been decided to use the more expensive and more difficult neutralization technique for the assessment of serum antitoxin levels in this study.

CHAPTER 4

THE POPULATION

4.1 Representativeness and Generalizability

The target, or reference, population is that population about which an investigator wishes to draw a conclusion (11). The results of this study are intended to yield information useful for the selection of a maternal tetanus immunization schedule for rural Sierra Leone. The target population is the women of child-bearing age in rural Sierra Leone, and the study group should be a representative sample of that target population. The accepted procedure for meeting the requirement of representativeness is the drawing of a random sample of the rural female populace, that is, a sample selected from the target population where all members of that population have an equal probability of being selected. This ideal cannot be reached within the practical limits of this trial; the experiment will be performed in one large chiefdom.

Cochran (10, page 5) states:

"Sometimes for reasons of practicability or convenience the sampled population is more restricted than the target population. If so, it should be remembered that conclusions drawn from the sample apply to the sampled population. Judgment about the extent to which these conclusions will also apply

to the target population must depend on other sources of information. Any supplementary information that can be gathered about the nature of the differences between sampled and target populations may be helpful."

The "supplementary information" of particular interest for the generalizability of this study would be serum antitoxin level, compliance rate, traditional birth attendant practices, and possibly, the prevalence of malaria and malnutrition. However, by its very nature, an efficacy trial will include unknown factors related to the self-selection of compliers that will reduce generalizability further. If a decision is made to implement the selected schedule in other areas of the country, continued monitoring of outcome should be done to ensure that the benefits remain within the expected limits.

4.2 Nongowa Chiefdom

4.2.1 Geographical Location and Demographic Information

The area chosen for this study is the Nongowa Chiefdom in the Kenema district of the Eastern Province. The population of this area is approximately 50,000, including 12,000 in Kenema Town and another 7,000 in three large villages. There are some 31,000 people living in at least 150 villages whose only access to medical care is by a long walk to the government hospital in Kenema Town.

The people of the chiefdom are primarily of the Mende tribe. Rural occupations include subsistence farming, lumbering, and diamond mining. Population density of the chiefdom is between 150 and 250 persons per square mile (59-98/km²).

Age and sex distributions (9) suggest that of the 31,000 rural inhabitants, 7,000 will be women of child-bearing age (15-44 years).

Crude birth rate in the district is 39 births per 1000 population per year or an expected 1210 live births per year in rural Nongowa Chiefdom. The estimated death rate (26) due to neonatal tetanus is 115 per 1000 live births or 143 neonatal tetanus deaths per year in the rural parts of the chiefdom.

4.2.2 Reasons for Selecting Nongowa Chiefdom

The reasons for selecting this chiefdom are:

- (i) The Maternal and Child Health (MCH) Programme of mobile clinics is scheduled to be expanded to this area in the next few years. This means that an area census will be done, contact with the villagers will be established and some health personnel and equipment will need to be obtained. Since all of these needs overlap the needs of the trial, a considerable practical advantage can be realized in arranging for these needs to be met, and the trial completed, before the introduction of the mobile clinic programme.

- (ii) The previous lack of rural medical programmes in this area means that it is unlikely that experimental contamination by prior exposure to tetanus toxoid has occurred.
- (iii) Coordination and monitoring of the trial is facilitated by the government hospital, communications, and central location of Kenema Town.

4.3 The Experimental Unit

4.3.1 Simple Random Assignment

The most subject-efficient experimental unit for this randomized trial would be a woman of child-bearing age. The number of women in each group will produce a number of live births, which will lead to a proportion of neonatal tetanus deaths. Individual women could be randomly allocated to receive each of the treatments. This simple randomized design would require an easily calculable number of subjects and would tend to balance out village and TBA effects.

4.3.2 Problems with Simple Random Assignment

Simple random assignment would cause a number of practical problems that would be very difficult or expensive to overcome.

- (i) The randomization procedure would require the vaccinators to follow a complex protocol. A randomized order of vials, or of labelled cards have been used in other trials to allocate toxoid types to groups in previous trials, but in these trials, done in densely populated areas, the vaccinators were supervised by senior study personnel. For this trial, vaccinators will have to work in the field with little supervision; reducing the number of decisions required should reduce the number of errors.
- (ii) The problems of error in allocation would be compounded by contamination errors in subsequent injections. The accidental injection of placebo to the intended three-dose group, of a third active injection to the two-dose group, or of active injection to the placebo group could seriously bias the results.

4.3.3 Randomized Allocation of Clusters

The decision has been made, on the basis of the above problems, to define the village as the experimental unit. Using a procedure detailed in the next chapter, villages will be randomly assigned to one of three groups, and all women estimated to be between the ages

of fifteen and forty-four will be offered the immunization schedule assigned that village. It is hoped that the loss of statistical efficiency will be compensated by the decrease in errors and contamination.

4.3.4 Implications of Randomization by Cluster

The loss of efficiency due to the randomization by cluster referred to above is manifested in two ways:

- (i) The number of immunized women required for this trial must be increased by a correction factor related to the intra-cluster correlation of the outcome variable. This detail will be discussed further in the sample size section.
- (ii) The number of degrees of freedom in the various statistical analyses are reduced from that which would pertain to the number of individuals to that of the number of villages.

4.4 Inclusion, Exclusion and Disqualification

4.4.1 Villages

Villages are defined as clusters of at least fifteen dwellings considered to be permanent residences, as opposed to the huts constructed for temporary shelter occasionally required during peak farming times. It has been estimated that there are at least 150

villages greater than this size in Nongowa Chiefdom. All villages in Nongowa Chiefdom are eligible as experimental units for this trial and as many will be entered as agree to participate. There are three villages of over 1,000 inhabitants in the chiefdom; if treatment centres are constructed in any of them before the start of the trial such that a tetanus immunization is attempted that does not follow the protocol of this study, then the village will be excluded. It is believed that this may have occurred in the largest village, but is unlikely to occur in others. Once the trial is progressing, tetanus toxoid will only be available through study personnel except in Kenema Town. No restriction will be put on the distribution of anti-tetanus serum required for treatment purposes. Kenema Town will be excluded for the above reasons; it is known that the government hospital has attempted immunization.

4.4.2 Individuals within Villages

The immunization schedule assigned to each village will be offered to all women of that village estimated to be within the ages of fifteen and forty-four. All who consent to participate will be entered into the trial. The following criteria will be used for disqualification after entry to the trial. Disqualification refers only to the removal of results from analysis; immunization and data collection will continue.

(i) Women whose initial antitoxin titre is above 0.001 IU/ml have probably had previous exposure to tetanus toxoids and will have their results excluded from analysis.

(ii) Women who are in the later stages of pregnancy during the immunization phase will have birth resulting from that pregnancy removed from the major analyses. One subsequent pregnancy by the same woman during the course of the study will be allowed. On the third immunization round the vaccination team midwife will examine each woman; any woman who is identified by abdominal palpation as being pregnant, will have details of that pregnancy recorded, and the results of that pregnancy will be disqualified. These births have been disqualified because:

a) births for a short time after an injection will not be affected by that injection. The length of this interval has not been well investigated, but it is at least one week, (52), and may be as long as four weeks (56). Stanfield and Gall (54) have demonstrated a lag from the rise in maternal antitoxin titres and the rise of the cord antitoxin titres.

b) the subsequent rise in titres is very steep and falls off quickly to a relatively stable level (recall Figure 2). It may be that births are "more protected" during the peak interval and, hence, if many births occurred during

the interval, the long term efficacy of the schedule could be overestimated.

4.5 The Size of the Experimental Population

4.5.1 Introduction

It is expected that the primary question will require the greatest number of subjects; sample size is discussed with reference to that question. However, comparable calculations will be done on the estimates for the secondary question, and the largest estimate of the number of subjects required will be used as the sample size requirement for all three groups.

As in the case of random assignment of individuals to groups, the between group difference to be detected (δ) in the randomization of clusters depends on the preset α and β levels and the hypothesized outcome proportions. Π_T and Π_C . However, an additional correction factor is required and this factor depends on the intra-cluster correlation (ρ) and the number of clusters (M, m) and the size of each cluster (N, n):

4.5.2 Definition of Terms

- (i) α is the risk of concluding that there is a difference in benefit of the schedules when in fact there is not. The α -level for this study has been set to the conventional 5 percent. The decision to use the two-dose schedule

will be the same if the schedule is as good as, or better than, the three-dose schedule. The test to be made then concerns only whether the three-dose schedule is better than the two-dose; hence, α is one-tailed.

- (ii) β is the risk of not achieving statistical significance if there is, in fact, a difference in benefit of predefined magnitude between schedules. β has been set to 5 percent, one-tailed.
- (iii) π_C is the expected death rate from neonatal tetanus in the three-dose group. In previous studies, the death rate in three-dose groups has ranged from 0 to 9 per 1000 live births; the higher figure included an unknown number of incomplete schedules. The estimate for this study has been taken as 0.005.
- (iv) δ is the statistically detectable difference between groups for given α , β and n . Or, conversely, if a preset δ is used to calculate the sample size required, differences smaller than δ will not be statistically significant: δ has been set in this study to 2%. Then, since $\pi_T - \pi_C = \delta$, $\pi_T = .025$.
- (v) M is the total number of clusters; m is the number of clusters sampled. By design, $m = \frac{M}{3}$, all available clusters will be assigned to one of the three groups.

- (vi) N_i is the number of sampling units in the i -th cluster, n_i is the number sampled. By design, $N_i = n_i$. Although until now, only women have been discussed, the appropriate secondary sampling unit is not mother, but rather, live born infant. The N_i , then, refer to the number of live births per cluster (village).
- (vii) ρ_i is the intra-cluster correlation coefficient of the i -th village. In general, (from Hansen and Hurwitz, (27), notation modified).

$$\rho_i = \frac{(\pi_i - \pi)^2 - \frac{\pi_i(1 - \pi_i)}{N_i}}{\pi(1 - \pi)}$$

Note: Other references on this topic with different approaches are:

1. other similar formulae - Cochran (10), Yamane (64)
2. relative efficiency approach - Kish (33), Cornfield (12,13)
3. where ρ has a lower bound at 0 - Yates and Zecopanay (65)

4.5.3 Estimating Sample Size Given Simple Random Assignment

The following formula can be used for estimating the required sample size per group. (From Colton (11))

$$n_{SR} = \left[\frac{Z_{\alpha} \sqrt{2\pi(1-\pi)} - Z_{\beta} \sqrt{\pi_C(1-\pi_C) + \pi_T(1-\pi_T)}}{\delta} \right]^2$$

under the null hypothesis $\pi_C = \pi_T = \pi$. Hence, using the previously defined rates,

$$n_{SRS} = \left[\frac{1.645 \sqrt{2(.005)(.995)} - (1.645) \sqrt{(.005)(.995) + (.025)(.975)}}{.02} \right]^2$$

$$= 497 \text{ births per group.}$$

4.5.4 Estimating the Sample Size Available

Dow and Benjamin (17) estimate a birth-rate in rural Sierra Leone of 40 per 1000 population per year. A very similar estimate of 39 per 1000 was obtained from the National Expanded Immunization Programme. Thus, in one year after the immunizations are complete and the exclusion period over, 1210 births would occur in the chiefdom outside of Kenema Town and the three large villages. If the goal rate of 80% immunization is reached, 968 births would be entered into the trial each year. Approximately twice the number of births required for simple random allocation would be obtained in three years.

4.5.5 The Correction Factor

For illustrative purposes, assume that all cluster sizes are equal (that is, $N_1 = N_2 = \dots N_M = \bar{N}$), then the correction factor is $1 + (\bar{N} - 1) \rho$. It can be seen that as ρ gets larger, the correction

factor expands very quickly. Fortunately for this trial, the very low and consistent Π_i 's expected should produce a small ρ .

In the case where the N_i are not equal, the formula expands to:

$$1 + \frac{\sum_{i=1}^M \rho_i N_i (N_i - 1)}{M \bar{N}}$$

Recall from 4.5.2 that ρ_i is a function of Π_i , N_i , and Π . It can be seen then, that an estimate of the correction factor requires an estimate of the distribution of Π_i 's per N_i , and of the distribution of N_i 's in M . These estimates are not presently available.

4.5.6 Determining Sample Size

In this trial, since the chiefdom population is fixed, increasing sample size means increasing length of follow-up. The length of follow-up will be determined as follows: an area census will be done in the chiefdom before the start of immunization. This will yield the number of eligible villages and the number of women per village. In Bo district, (26) as part of another study, several areas have been carefully surveyed and exposed to the MCH mobile team programme. Part of this programme included a three-dose tetanus toxoid schedule. Records from as many of these villages as are available will be examined and the rates of neonatal tetanus deaths per year per village and births

per woman per year will be applied to the distribution of village sizes and number of women per village in Nongowa Chiefdom. The correction factor will be calculated and multiplied by the size calculated in 4.5.3. The length of follow-up will then be determined by the length of time needed to obtain the number of births required.

4.5.7 β Analysis

As with any sample size calculation, the observed data are likely to differ from the hypothesized and assumed values used for the calculation. At the end of the trial, the observed proportions and differences, and the actual number of births will be used to calculate the statistical power ($1 - \beta$) that applies to the results of the trial.

CHAPTER 5

PRE-IMMUNIZATION PROCEDURES

5.1 Introduction

For economic reasons an effort has been made in the design of this trial, to utilize either existing personnel, procedures and equipment, or those that will be required for the subsequent introduction of the Maternal and Child Health mobile clinics into Nongowa Chiefdom. The MCH clinic system will require routing information, trucks and coldboxes, and personnel for operation and maintenance of equipment. These and other materials will be available from the trial. The area census needed by the MCH team can be superimposed on the initial and follow-up interviewing needed by the trial.

5.2 The Mobile Teams

5.2.1 MCH Teams

Although the MCH clinics will not be in operation until after the trial, some aspects of the design are discussed with reference to the MCH needs. Thus, for illustrative purposes, the following is a general description of the MCH plan. The mobile MCH programme routes a truck-borne team of health workers to predetermined assembly points in a chiefdom at least once every three months. The assembly

points are located centrally to a number of villages such that the furthest distance to be walked by a villager to the assembly point is three miles. A MCH mobile team consists of one nurse-midwife, one MCH aide, one advance man and a driver. Before the team arrives at an assembly point, the motorcycle-equipped advance man rides to the villages covered by that assembly point to inform villagers of the time of arrival and location of the team.

5.2.2 EDCU Teams

(i) The Assessment Teams

A Senior Operations Officer, an Assistant Operations Officer, and teams of assessment personnel are based in the Endemic Disease Control Unit headquarters at Bo, a large interior town in the Southern Province. The teams are responsible for the data gathering and preparation required by the MCH mobile clinics prior to their introduction to a new chiefdom. An assessment team consists of a team leader, three assessors and a driver.

(ii) The Vaccination Teams

The Endemic Disease Control Unit also maintains mobile vaccination teams in each province. These teams are intended to administer the vaccinations prescribed by the Sierra Leone National Expanded Immunization Programme in areas not covered by either the MCH mobile teams

or by static treatment centres. Each team consists of a team leader, two vaccinators, an advance man, and a driver. The EDCU also maintains over 200 other staff besides the teams mentioned here. The vaccination teams will each require the addition of one trained nurse-midwife during the immunization period; these will be obtained from EDCU staff.

5.2.3 Team Utilization

The Endemic Disease Control Unit assessment teams will be responsible for the initial area census, the data gathering at the time of initial census, and for the follow-up assessment. Additional staff and equipment will accompany the assessors at follow-up to obtain outcome blood samples. The EDCU vaccination teams, supplemented by one nurse-midwife each will administer the injections, and obtain blood samples at the time of immunization.

5.3 Mapping the Chiefdom

If a new map of Nongowa Chiefdom is not yet available from the recent aerial survey, the 1963 version will be used and, if obtainable, the aerial photographs will be used to update village locations, roads and paths. This map can be used to begin to plan the routing for the MCH mobile team, but its first use is as a guide for the EDCU assessment

team responsible for the area census. This team will verify the existence and location of the villages shown, and also ascertain whether new villages or new roads and paths have developed since the aerial survey. Although all villages originally on the map will already have been assigned code numbers, additional codes will be available to each team to assign to villages not on the map. Assessors are required to use the village codes to identify the data on individuals by their village of residence.

Upon the return of the advance teams, the map will be updated and code numbers will be cross-referenced by local village name and by map coordinates. This should sufficiently guarantee against the subsequent confusion of villages by the several teams involved in the study. The final map can then be used for routing the EDCU vaccinators, for determining appropriate locations for the mobile clinics and for information required for randomization.

5.4 Initial Contact

5.4.1 Local Permission

The procedure to be followed is based on the usual introduction of the MCH programme to a chiefdom. First, the permission of the Paramount Chief will be sought. The reasons for the trial, the blood sampling, and all injections will be fully described. With the support of the Paramount Chief, a subsequent meeting with the Section Chiefs should gain their consent. When this is obtained,

personnel from the EDCU accompanied by representatives of the Paramount Chief (usually policemen) will travel to all villages of the chiefdom to inform the villagers of the trial, to explain its potential benefit and harm, and to describe the injections and blood sampling procedures.

The women will be informed that they are free to refuse the injections.

5.4.2 The Area Census

At the same time, the assessment teams will collect census information. The usual MCH-required data will be obtained; this includes a population count by sex and age categories, morbidity and mortality rates with particular emphasis on the diseases of childhood, and fertility and childbearing information of the women of the village.

For reasons described later in this chapter and in the Measurement chapter, the following additions and modifications will be made:

- (i) Data from villages and from women within villages must be individually and uniquely identified. The procedure used must continue to uniquely identify women and villages on several subsequent occasions.
- (ii) Questions necessary for the differential diagnosis of neonatal tetanus death will be added.

- (iii) The procedures used to ascertain infant mortality, particularly neonatal tetanus mortality, must closely follow the protocol described later.
- (iv) A time-frame for the assessor to investigate will be specified.


5.4.3 Initial Assessment Blindness

The randomized assignment of villages to treatment groups will not have been made at the time of the area census; hence, the advance team will be unaware of the eventual treatment status of the villagers they interview. It might be anticipated that problems with compliance would occur if the villagers were aware that two doses may be sufficient to immunize them. To ensure that both villagers and assessors remain unaware of the two-dose/three-dose nature of the question, the advance team will only be informed that this is a trial of different three-dose preparations.

5.5 Identification Procedures

5.5.1 Village

The details of village identification were described in the mapping section. The village code will consist of a letter denoting the initial assessment team and a two digit number. The team identification is required for cross-checking and for making codes available for each team (without overlap) for villages not on the original map.



As mentioned before, village codes will be cross-referenced by local names.

5.5.2 Individuals within Villages

Recommended by Morley (40), a reliable and economic way of keeping family health records in a developing country is to supply the mother with durable cards for herself and her family, with a sturdy sealable plastic bag for storage. She is then asked to bring the cards to all subsequent contacts with health teams.

In this trial, some of the anticipated sub-analyses, particularly those of compliance and serum antitoxin levels, require the unique identification of each study subject for follow-up. Hence, at the time of the initial census, each woman estimated to be within the ages of fifteen and forty-four will be given a card on which is marked a four-digit code number, her name(s) and the village name and identification code. It will be stressed to each woman that these cards are not to be given to anyone else, and that it is essential to bring the card to every health care contact. The area census data and all subsequent data will be identified by the combination of the village code and the four-digit code on the card.

5.5.3 Vaccines

Bottles of vaccine will only be identified by the name of the village in which it is to be used, and by a code referring to the

vaccinating team assigned to administer it.

5.5.4 Cross-Checking Identification

After each round of injections, all data will be gathered at the Kenema Town study office and cross-checked to ensure that the schedules have been administered appropriately. The above identification procedure allows several levels of cross-checks. The EDCU log data will be used to check that the women of each village received injections from the bottle assigned that village.

5.6 Randomization

5.6.1 Introduction

As previously discussed, practical considerations do not permit the randomization of individual women into treatment groups. The plan, then, is to randomize all chiefdom villages into three groups, and offer the residents of each village the immunization schedule of the group to which the village was assigned.

5.6.2 Procedure

To implement this plan, and to minimize imbalance, the area census data obtained by the advance teams will be used to rank villages by the number of women of child-bearing age that are resident in each village. A list of the size-ranked villages will

be produced. If there are two or more villages of the same size, the order of villages within these tied ranks will be determined randomly; the villages in each tied rank will be assigned equiprobable numbers and then entered in the list in the order in which these numbers are found in a random number table. Then, starting with the largest three villages, each three villages in order will be selected. The group assignments of the members of each of these triplets will be determined randomly. There are six ways in which the three villages can be allocated to the three groups, see Table 1.

TABLE 1

PERMUTATIONS	GROUPS		
	PLACEBO	2-DOSE	3-DOSE
1	Village I	Village II	Village III
2	I	III	II
3	II	I	III
4	II	III	I
	III	I	II
6	III	II	I

The randomization procedure is defined as follows: if the next number in a sequence of one digit numbers in a random number table is between one to six inclusive, the group assignment of all three

groups will correspond to the permutation in Table 1 beside which the number is listed. If the next number in the sequence is 0, 7, 8, or 9, it will be ignored and the next in sequence selected.

Throughout this procedure, the villages are identified only by village code number. Vaccine codes appropriate to the group assignment can then be transferred to the maps needed by the EDCU for routing and the administration of the appropriate immunization schedule.

5.6.3 Justification of the Randomization Procedure

The procedure described above resembles a matching procedure, but has been done only to ensure a size balance between groups. The usual reason for matching is to attempt to reduce variability and thus increase precision. Matched samples should always be compared using matched analyses. These analyses make the comparisons between matched units, and if the matching variables were indeed confounders, the tests will be more efficient than if the matching had not been done.

In this trial, the villages have been grouped only on size, they are not truly matched; it may be inappropriate to attempt to gain the increased efficiency by using matched analyses. However, it could be suggested that the size of villages may be related to certain traditions and thus to outcome; so an adjustment procedure, or post-stratification, for village size is included in the analysis

(see Chapter 8).

5.6.4 Stratification Variables

Introducing stratification variables to the above procedure would require that the restrictions on size be relaxed to broader categories, and thus increase the risk of a large size imbalance. However, it is possible to adjust for between-group differences in the distributions of variables in the analysis of data. Since it is not possible to adjust for ~~insufficient~~ insufficient sample size, it has been decided to retain the size balancing procedure and to leave other variables for subsequent statistical adjustment.

CHAPTER 6

THE MANOEUVRE

6.1 The Immunization Schedules

NOTE: In the ensuing discussion it is necessary to discriminate the term "dose" which will refer to an injection of tetanus toxoid, from the more general term "injection" which may contain either tetanus toxoid or placebo.

6.1.1 Introduction

At the end of the immunization phase, in the three-dose group, there is likely to be a distribution of people with 0, 1, 2, or 3 injections, despite efforts to attain a very high proportion with three injections. The selective factors that operate to produce non-compliance need to be controlled in this trial. To do this, all three groups will be offered three injections in an identical fashion. The necessary comparisons between groups can then be made with suitable adjustment for number of injections.

6.1.2 The Interval Between Injections

The serum antitoxin level study by Dhillon and Menon (15) suggests that the interval between two doses of concentrated adsorbed toxoid be at least twelve weeks. Although the recommended intervals in western countries are longer, Sierra Leone (and India (56)) has been administering its three-dose immunization with intervals between injections of four to six weeks. Newell's (43) randomized trial in Columbia used intervals that varied, apparently unsystematically, from six weeks to more than one year. The interval for this study has been selected to attain the minimum twelve week interval in the two-dose group without unduly lengthening the trial. For these reasons the interval between injections in the three-dose group will be six to eight weeks.

6.1.3 The Two-Dose Schedule

The two-dose schedule consists of two 0.5 ml intramuscular injections, administered twelve to sixteen weeks apart, of a concentrated tetanus toxoid, titrating 25 Lf per dose and adsorbed on 3.86 mg aluminum phosphate. While it is possible to increase both concentration and adsorbant, these amounts represent the maximum permissible for licensing in Canada (31). Six to eight weeks following the first injection of toxoid, a second injection of an inert placebo will be administered to the two-dose group, yielding the three injections at six to eight week intervals

necessary for compliance comparisons.

6.1.4 The Three-Dose Schedule

The three-dose schedule consists of three 0.5 ml intramuscular injections, administered at six to eight week intervals, of a commercially available tetanus toxoid, titrating 10 Lf units per dose, adsorbed an aluminum phosphate. The dose and potency of this toxoid is the same as that reported by Berggren in Haiti(4).

NOTE: Both tetanus toxoids will be prepared from the same parent toxoid.

6.1.5 The Placebo Schedule

The placebo schedule consists of three 0.5 ml injections, administered at six to eight week intervals, of a 0.9 percent solution of sodium chloride.

6.2 EDCU Vaccination Team Procedures

6.2.1 Introduction

After the area census and the randomization of villages to groups is complete, the EDCU can schedule and route its mobile teams into the interior to administer the prescribed injections.

6.2.2 Immunization Procedures

Vaccination trips will be scheduled around peak farming times, and the villagers will be informed of the arrival of the team by the advance men. By not competing with other time demands on the villages, compliance should be increased. On each trip to each village, the vaccination team will offer the assigned injections to all eligible women of the village. The vaccine code and the date of immunization will be entered on the woman's card and the identification codes on the card will be recorded in the team log along with vaccine code and date.

At the time of the first injection a 5 ml blood sample will be drawn and stored. This sample will be identified with the village code and the woman's identification code.

All women will be examined by the nurse-midwife at the time of the third injection, and details of pregnancies will be recorded.

Three rotations through the villages will complete the immunization schedule. From previous experience in Bo District (26) where women were requested to appear at assembly points for injections, 80% of the eligible women will accept the first injection, 65% the second, and 40% the third. It is hoped that these compliance rates can be improved considerably by taking the teams to the actual villages, by scheduling trips at convenient times, and by dispatching

drivers and advance men to invite missing women in from the fields, or if necessary, sending vaccinators out to the fields to administer the injections.

6.2.3 Post-Trial Removal of Coded Data

So that the MCH programme will have accurate records for future use, the data from the vaccination team log will be transferred to a new set of cards at the trial headquarters. These new cards will contain no coded data. These cards will be exchanged for the trial cards when the MCH mobile teams are introduced to the chiefdom.

CHAPTER 7

MEASUREMENT

7.1 Introduction

7.1.1 General Procedures

The EDCU assessment team will obtain at the time of the initial area census, the necessary baseline data required by the trial, identified by individual, as well as the usual grouped health and census data. A similar rotation, but with the addition of blood sampling, through the villages at the end of the trial will provide the outcome data necessary for the determination of relative efficacy. Other required data will be obtained at the times of the various immunizations.

7.1.2 Maintaining Blindness

The assessment teams will not be informed that they are obtaining data of the efficacy of a reduced dose tetanus immunization schedule. They will not know the group assignment of the villages.

The initial area census questions concerning the incidence and prevalence of target diseases will again be superimposed on the trial questions at the time of the follow-up assessment. This will serve to disguise the experimental intent of investigating a differential

response to the three schedules in terms of reduced tetanus deaths. Additionally, the follow-up census will be used to provide descriptive data for the introduction of the MCH clinics.

7.2 Non-Interview Data

7.2.1 Identifiers

The necessity of, and the procedures for, the individual identification of women, villages, and toxoids have been discussed in the previous chapter.

7.2.2 Maternal Immunization and Compliance

Compliance, in this trial, refers to the adherence of each woman to the immunization schedule on which she was started. A complete schedule (i.e. complete compliance) consists of all three assigned injections. The degree of non-compliance is measured by the number of injections not received. The number of injections per individual will have been recorded in the EDCU log. This information will be abstracted from the log and will be used to identify compliers for the efficacy questions.

7.2.3 Serum Antitoxin Levels

Immediately before the first injection, and at the follow-up assessment, 5 ml of blood will be drawn from each study subject. The blood will be identified, prepared for storage, and stored in

the truck refrigerator until it can be transported to Kenema Town. From Kenema Town, it will be flown to Freetown, and if necessary, from there to the test laboratory. The mouse-neutralization procedure will be done to ascertain the antitoxin level in the blood.

Serum antitoxin level is used as a disqualification criterion, as the outcome of one of the secondary questions.

7.3 Interview Procedures

7.3.1 Interview Format

The EDCU assessment personnel will be trained to follow a pattern of questioning which starts with general less obtrusive questions and follows with the more specific detailed ones. For instance the order of questioning about infants will proceed from live births, to infant morbidity, to infant mortality, to the age at death and the details of feeding and posture required for the differential diagnosis of neonatal tetanus.

Although a specific name for neonatal tetanus exists in every dialect in the country, the name will not be used in questioning. If the respondent suggests the name as the cause of death, the assessor must push to ascertain the required criteria. The data collection form to be used will serve to remind the assessor of the order of questioning and the details required.

Since it is likely that the assessor may have to simultaneously interview several study and non-study subjects, special training and

practice in directing the discussion and keeping information separate must be arranged. The order of questioning must be maintained even in this circumstance so as not to unnerve subjects who have not yet responded by revealing to them some of the more invasive questions. The requirement is, then, to obtain answers from each of the respondents before proceeding to the next area of questioning.

7.3.2 Initial Testing of the Interview Procedures

Health workers familiar with the dialect and customs of the district will design the preamble, phrasing and style of questioning within the ~~ordered~~ framework described in the last section. Practice interviews will be done on conveniently available area mothers to identify such problems as ambiguity, insensitivity or offensiveness, omitted details, and the like. Changes will be made as necessary. When the procedures are satisfactory in this situation a more formal pretest of the primary outcome measure will be under-taken.

7.3.3 The Pre-Test of Neonatal Tetanus Death Ascertainment

The admissions records of the Kenema Town hospital will be examined, starting with the most recent. All neonatal tetanus admissions will be recorded until 100 are obtained and all other

infant admissions in the same time period will be listed. An equal number of the non-tetanus admissions will be randomly selected from the list. If this time period starts to exceed the planned length of the trial, additional cases will be obtained from the treatment centres at Panguma or Blama, in neighbouring chiefdoms, rather than going back further in the Kenema records. The blinded members of the assessment teams will attempt to locate the mothers of the admitted infants and will interview them using the assessment procedure. An unblinded observer will subsequently seek additional information, particularly when the assessor was unable to obtain the correct or complete answer. This information may be necessary to modify the procedures if increased accuracy is required.

It is expected, given the rapid onset and distinctive symptoms of neonatal tetanus, that very few false positives will be recorded. Nor is it likely concomitant illness will disguise the symptoms and cause false negative reports. Non-fatal cases may be missed, but the untreated case fatality rate is very high, and since there is little reason to hypothesize a difference in case fatality rates in the groups, these missed cases are unlikely to affect the results. For these same reasons, the original question was phrased in terms of reduction in deaths rather than reduction in disease.

Ignoring non-fatal cases, modifications will be made if agreement is less than 80%.

7.4 Interview Data

7.4.1 Live Births

These data are required as the denominator portion of the outcome variable and refer to those neonates at risk of tetanus death. Since the shortest incubation period reported in the literature is two days, it is assumed that infants dying before they are two days old would not have died of tetanus infection. Hence, a live birth refers to an infant who lives for at least two days, and who has cried and sucked normally during that time.

Birth data will be collected by interview at both the time of initial census and at the end of the trial. Numbers of births are a standard part of the area census, but, for the purposes of the trial, it is necessary to identify births by mother and by village, so that the births can be linked to the immunization and compliance data of the mother.

7.4.2 Neonatal Tetanus Death

The primary outcome measure of the trial, neonatal tetanus death, is considered to have occurred if the following factors are present:

- (i) The infant cried and sucked normally after birth, but some days later was unable to suck.

- (ii) That risus sardonicus can be described or mimicked by the mother,
- (iii) The typical spasms (flexion of the arms, clenched fists, extension of the legs, plantar flexion of the toes) can be described or mimicked by the mother,
- (iv) That the death occurred within twenty-eight days after birth.

This list represents the preferred definition, but it is unlikely that the respondents will have the precise time sense required by some of the factors. These factors will be modified to suit the local situation when the interview procedures are designed or during the initial testing. Once a satisfactory pre-testing is completed no further modifications will be made.

An illustration of the type of modification might be the substitution of "before the infant was named" for "within twenty-eight days" since babies are commonly not named until several weeks after birth. Since it may be unlikely that the mother is questioned alone, others may contribute appropriately to the diagnostic information so perhaps where "mother" occurs in the definition, this may need to be changed to "mother and certain relatives".

Deaths are required to be recorded by individual mother so that immunization and compliance data can be linked to outcome.

7.4.3 Non-Tetanus Deaths

All infant deaths during the course of the study will be recorded during interviewing for the purpose of determining both initial and ongoing comparability of the groups for non-tetanus related events, and to ensure that the overall infant mortality ratio will fall with a decrease in neonatal tetanus deaths.

7.4.4 Traditional Birth Attendant

The name of the "granny" attending each birth will be obtained. If an unexpected number of tetanus deaths occur in either group, subanalyses will be done to examine whether they are TBA related.

7.4.5 Items Associated with Risk in Other Studies

- (i) Sex: Sex of the infant
- (ii) Age: Age of the women will be obtained using the broad age categories and estimation procedures presently used by census takers, since age in years or birth date are unlikely to be known.
- (iii) Previous pregnancies: During the initial interview the number of previous pregnancies will be obtained.
- (iv) Season of birth: An estimate of the birth data will be obtained.

In reviews of neonatal tetanus, it is usually stated that tetanus deaths occur much more frequently in males than females. (e.g. Beaty (2) reports a male:female ratio of 2.5:1). Both Schofield (52) and Newell (43) report approximately equal numbers of male and female deaths. There would seem to be an equal probability of infection of males and females; the difference in rates may result from some sort of reporting bias. However, sex data will be collected, and included in the analysis in case there is a true difference in susceptibility, or a difference in traditions that would increase risk of infection in one sex.

An increased risk of tetanus infection with age, number of previous pregnancies and season has been reported in other studies. These data will be collected even though there are strong alternate explanations for each of the above mentioned associations. Tompkins (57) described a seasonal variation. This was a hospital admissions study and the small changes could have been related either to seasonal accessibility changes of the hospital or seasonal time demands on the parents. Berggren and Berggren (3) describe the pregnancy and age relations to tetanus infection; that is, that older or more often pregnant women have a greater chance of tetanus infection of their children. This conclusion was clearly confounded with the number of events at risk which had occurred. While it may be true that the older or more often pregnant woman is more likely to have had an infant tetanus death, it does not imply that the risk

is increasing with age or pregnancy and that the woman is now at greater risk than she was earlier.

CHAPTER 8

ANALYSIS

8.1 Analysis of the Death Outcome Questions

8.1.1 Introduction

The research questions require a test of the relationship of the three categories of treatment to the two categories of outcome. If a relationship between treatment and outcome exists, then a further examination will be done to identify which of the three treatments effect outcome. Other variables that are potentially related to outcome need also to be accounted for, so that any effect of these variables is not "confounded" with the effect of treatment.

8.1.2 The Contingency Table

The variables of interest for this analysis all consist of counts of occurrences in two or more classes of events. For example, the number of occurrences of male and female infants can be counted and placed in two classes or categories of the variable "sex". The units of interest (live births) can then be cross-classified by each of these sets of categories (alive-dead, male-female, etc.). The resultant contingency table is displayed in Table 2.

TABLE 2

CONTINGENCY TABLE

TREATMENT GROUP	VILLAGE SIZE	PRE-INTERVENTION TETANUS MORTALITY RATE (quartile)	SEX OF NEONATE	OUTCOME	
				TETANUS YES	DEATH NO
TWO-DOSE TETANUS TOXOID SCHEDULE	SMALL	I	M		
			F		
		II	M		
			F		
	INTERMEDIATE	III	M		
			F		
		IV	M		
			F		
	LARGE	I	M		
			F		
		II	M		
			F		
	SMALL	III	M		
			F		
		IV	M		
			F		
THREE-DOSE TETANUS TOXOID SCHEDULE	SMALL	I	M		
			F		
		II	M		
			F		
	INTERMEDIATE	III	M		
			F		
		IV	M		
			F		
	LARGE	I	M		
			F		
		II	M		
			F		
	SMALL	III	M		
			F		
		IV	M		
			F		
PLACEBO SCHEDULE	SMALL	I	M		
			F		
		II	M		
			F		
	INTERMEDIATE	III	M		
			F		
		IV	M		
			F		
	LARGE	I	M		
			F		
		II	M		
			F		
	SMALL	III	M		
			F		
		IV	M		
			F		

8.1.3 Description of the Analyses

The analyses of this multi-dimensional contingency table will be done using loglinear models. (General references: Bishop et al (5), Fienberg (24), Upton (58), BMDP (16)). The advantages of this technique in analyzing multi-dimensional cross-classifications over alternative techniques have been summarized by Fienberg (24, page 1).

In this technique, the logarithm of the expected cell frequency is written as an additive function of main effects and interactions. When all main effects and all possible interactions are included in the model (the "saturated model") then the expected cell values derived from that model will perfectly match the observed cell values. Removal of some of the interactions may not cause the predicted cell values to deviate significantly from the observed cell values. Significant deviation is determined by a χ^2 comparison of the observed and expected values, on degrees of freedom equal to the number of terms removed from the model. When the removal of one of the terms from the model does cause a significant deviation from the observed values, then that factor or interaction "has an effect" and must be retained in the model. For instance, if the removal of the treatment-by-outcome interaction causes a significant deviation, then that interaction has an effect; or, a difference in outcome exists between treatment groups. If all interactions can be removed from the model without a significant difference between predicted and observed cell values, then one is left with

the "model of complete independence", that is, none of the variables has an effect on any other variable.*

In addressing the primary question, one needs to test all interactions which contain outcome. For example, the treatment-by-outcome interaction tests for a differential effect of treatment or, a treatment-by-sex-by-outcome interaction would imply a differential effect of treatment on outcome, depending on the sex of the child. The treatment-by-sex interaction, while it may be interesting, does not address the primary question.**

8.1.4 Variables in the Primary Analysis

- (i) Outcome: Categorized into number dead of tetanus and number not dead of tetanus, these two counts sum to the number of live births. (Note: specific definition of live birth, Chapter 7.)
- (ii) Treatment: All villages are placed in one of the three categories corresponding to immunization schedule.

* Note 1: This general description is for illustrative purposes. Following a strict decision rule on the removal of terms may cause a slightly different model to be selected depending on whether one works up from complete independence or down from saturation. These borderline terms should be investigated for their potential contribution to the interpretation of the question addressed.

** Note 2: Upton (58) suggests, in cases where one is not interested in the relationship between explanatory variables, that the various response variable by explanatory variable interactions be tested for inclusion with all explanatory variable interactions included in the model.

- (iii) Sex of Neonate: Some studies have reported an increased occurrence of neonatal tetanus in males. Hence the two levels of sex are included in the contingency table.
- (iv) Pre-intervention Tetanus Mortality Rate: The neonatal tetanus death rate data from the area census will be categorized and included in the analysis. No information is presently available to suggest the number of categories needed for this variable. The initial approach will be to divide the rates into quartiles and analyze the contingency table using these quartile divisions. The table will be re-analyzed after moving the cut-points or using any natural cut-points suggested by the distribution of initial rates. If the results are insensitive to these changes, one can have confidence in the quartile divisions.
- (v) Village Size: Villages will be categorized into one of three levels according to the total population in each village at the time of the initial census.

8.1.5 Other Variables

The number of data points available (villages) limit the number of variables to test for inclusion in the model. If the death rates in the two toxoid groups are reduced as hypothesized, it

will be difficult to relate any other variables to differential outcome. If, however, either one or both groups significantly exceed the hypothesized rates, several variables are of interest in examining the unexpected result. These are TBA, TBA experience (number of previous deliveries), geographic location, incidence of malaria, prevalence of malnutrition, and other variables which may suggest themselves during the course of the trial.

8.1.6 Reduction in Total Deaths

As mentioned before, it is desirable to ascertain whether the total neonatal mortality rate will be reduced with a reduction in neonatal tetanus deaths. It could be argued that the lives saved from tetanus are those that will be lost to another cause (a competing risk argument) and thus it is not useful to implement the anti-tetanus programme. If the two-dose schedule is shown to be efficacious, this question will be addressed by a similar analysis to that just described, except that the outcome categories will be replaced by "all neonatal deaths" and "alive". If the results of this analysis parallel the results of the previous one, the competing risk argument becomes less tenable.

8.2 Analysis of the Serum Antitoxin Level Question

8.2.1. Introduction

This question requires a test of the relationship of two of the categories of treatment, the active toxoid schedules, to the

outcome variable, serum antitoxin level. Serum antitoxin level is a continuous variable; the analysis to be used will be multiple linear regression (General references: Kleinbaum and Kupper (34), Draper and Smith (18)) rather than discrete multivariable technique described in the previous section.

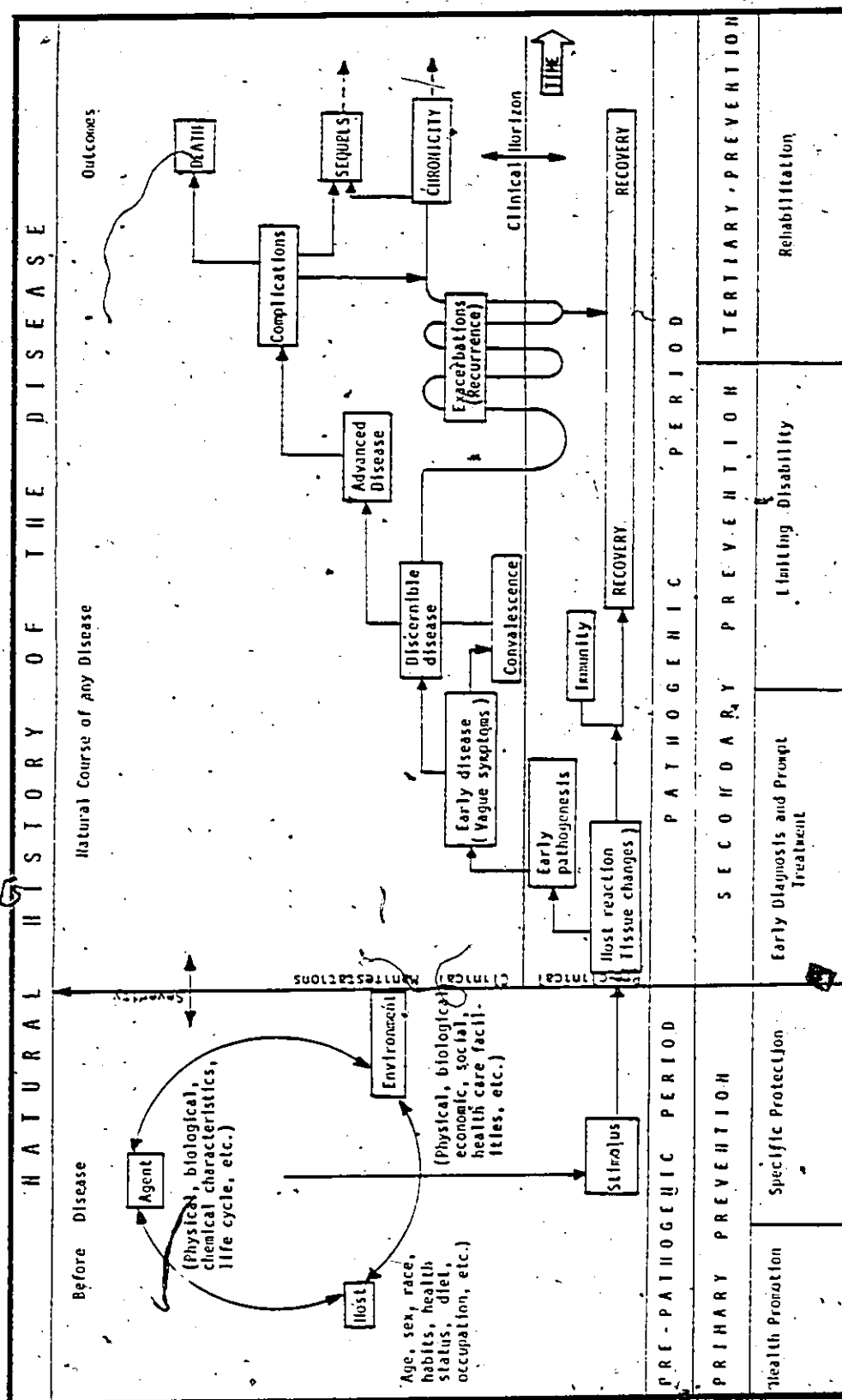
8.2.2 The Analysis

The question of interest is whether the treatments relate differentially to outcome. Using the multiple linear regression technique, one can obtain a prediction equation that indicates how the scores on the independent variables (treatment group, age, and other potential confounders such as geographical location, incidence of malaria, prevalence of malnutrition) can be weighted and summed to obtain the best possible prediction of the dependent variable (serum antitoxin level) for the sample. Variables which do not add substantially to prediction accuracy can be deleted from the prediction equation. If, after potential confounding variables have been included in the model, the addition of the treatment variable does not add substantially to prediction accuracy, then one can conclude that no differential effect exists between the two-dose and three-dose schedules with respect to serum antitoxin levels.

APPENDIX A

NATURAL HISTORY FLOW CHART: GENERAL FORMAT

Courtesy of: Dr. Lucía Yañez
Design, Measurement
and Evaluation
McMaster University



APPENDIX B

SUMMARY OF TECHNIQUES FOR DETERMINING SERUM TETANUS ANTITOXIN LEVEL

1. MOUSE NEUTRALIZATION TEST
2. HEMAGGLUTINATION TEST

Courtesy of: Dr. Mark McDermott
Department of Pathology
McMaster University

MOUSE NEUTRALIZATION TEST FOR ANTITOXIN TO TETANUS TOXIN

The purpose of this test is to determine whether or not a particular serum sample contains antibodies (antitoxins) directed against tetanus toxin and, if so, what the relative concentration of these antibodies might be.

Reactions between antitoxin (antibodies) and tetanus toxin (antigen) cannot be discreetly observed and therefore reaction indicator system is necessary. The following indicator system is based upon the capacity of anti-tetanus antibodies to protect mice from death following subcutaneous injection of either tetanus toxin.

Prior to conducting the test, the minimum amount of toxin which will cause 100% of injected mice to succumb within 96 hr is determined. To test a suspect serum sample for antitoxin activity, this minimum lethal quantity of toxin is mixed, in a tube, with a known volume of the serum. If antibodies are present in the serum, they will combine with the toxin. Toxin combined with antibodies is not capable of killing the mice i.e. the toxin is neutralized. When this mixture is injected into the mice, if antitoxin antibodies were present in the serum all mice will survive. If antitoxin antibodies were not present or present in very low quantities (therefore

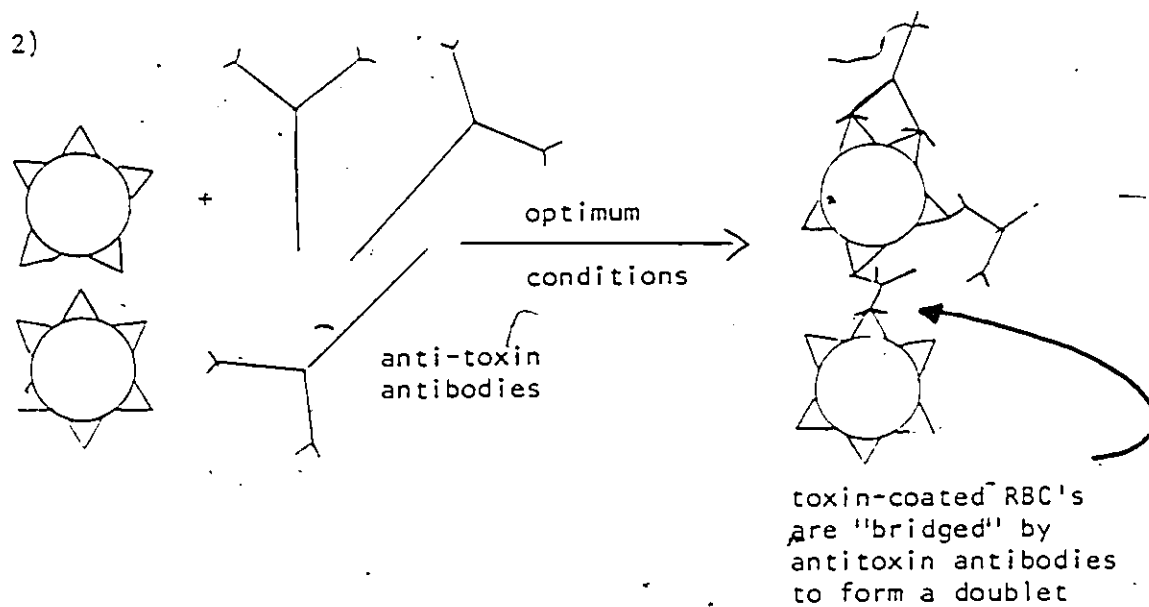
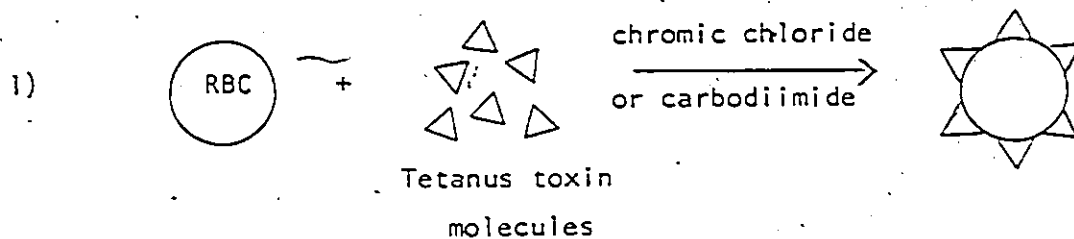
not combining with all toxin molecules) all mice will die. The source of the antitoxin (i.e. human, equine, bovine, etc.) has no effect on the assay; antibodies from these sources function in the same way as do antibodies from mice.

In most serum samples there is an excess of antitoxin antibodies, i.e. only a small portion of the antibodies are used up in completely neutralizing the toxin. Thus, the survival of the mice only indicates that antitoxin antibodies are present and says nothing about their concentration. To determine the relative (not absolute) concentration of antitoxin antibodies in the serum, the serum sample is first diluted with a buffer, then mixed with the toxin and injected. By making the dilution great enough, a dilution will be reached at which too few antibodies are present to neutralize the toxin and the mice die. For example, this dilution might be around 1 part serum to 10^3 parts buffer and the serum would be said to have a titre of 1:1000. Various serum samples can be compared to each other in terms of titre, i.e. 1:1000 vs 1:5000, etc.

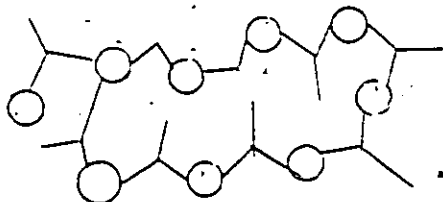
HEMAGGLUTINATION TEST FOR ANTITOXIN TO TETANUS TOXIN

The purpose of this test is identical to the mouse neutralization test; it is, however, considerably less expensive and much quicker (2-3 hr vs 96 hr).

The indicator system used is red blood cells (RBC's). The tetanus toxin (or toxoid derivative) is chemically coupled to the surface of the RBC's using either carbodiimide or chromic chloride. This procedure does not damage the cells and they remain monodisperse i.e. not stuck together as doublets, triplets, etc. Each antitoxin antibody molecule is bivalent and thus is capable of binding two molecules to toxin. Under the proper conditions, antitoxin antibodies may bind between two toxin-coated cells in a bridging manner (see diagram). Thus, a simple test for the presence of antitoxin antibodies would be mixing toxin-coated RBC's with the suspect serum. If doublets or triplets of RBC's were visible under the microscope, antibodies must have been present in the suspect serum.



If a large, standard number of toxin coated RBC's are added to a large number of antitoxin antibody molecules a large, lattice-like array of "bridged" RBC's occurs.



This is called agglutination and looks like congealed blood in a test tube, i.e. a microscope is not needed. The agglutination reaction can also be used in the following way. If toxin coated RBC's are mixed with antitoxin antibodies and this mixture placed in a V-bottom dish (dia. ~ 0.5 cm) the agglutination RBC's cannot roll down to the bottom of the dish as they would in the absence of antibodies.

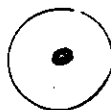


no antibodies



antibodies present

The RBC's look like a red spot in the bottom of the well if they are not agglutinated and do not appear this way if agglutinated.



from above

no antibodies

In each of these tests (the V-bottom well is most common) agglutination indicates only the presence of antitoxin antibodies and not their concentration. Analogous to the mouse neutralization test, the antibodies can be diluted until agglutination does not occur, and hence, various sera can be compared in terms of agglutination titre.

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