CONCEPTS OF LOUSE-BORNE TYPHUS CONTROL IN DEVELOPING COUNTRIES: THE USE OF THE LIVING ATTENUATED E STRAIN TYPHUS VACCINE IN EPIDEMIC AND ENDEMIC SITUATIONS

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INTRODUCTION

Louse-borne or epidemic typhus fever is without doubt one of the great epidemic diseases of mankind whose ebb and flow through the centuries has been important in the molding of human destiny (1). Much of our knowledge about typhus fever has come from experience in the unique setting of the temperate north of Europe (1-16) where its relative importance has declined as living conditions have improved over many years. Indeed, in recent times the epidemic potential of typhus in Europe has been realized primarily in the wake of the catastrophic disruptions caused by wars. Our attitude about relative importance, epidemiology, approaches to control and level of research support has been influenced strongly by the trends in modern, advanced Europe.

However, there is another face to typhus, hardly mentioned in our textbooks, largely ignored by investigators and treated as an unwanted step-child by many health authorities, which may be readily seen today in the less developed areas of the world where huge numbers of human beings still live continuously under conditions which most of us would regard as catastrophic and where typhus is a problem. Indeed, at present typhus is endemic to a greater or lesser degree on all continents except Australia and it is epidemic on at least one.

An attempt, albeit brief, will be made here (a) to construct a conceptual framework of the natural history of typhus infections and then, in the context of this framework, (b) to describe the actual state of typhus in some developing countries, (c) to consider

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the applicability and limitations of available control methods under the conditions which exist in such areas and (d) to illustrate briefly how one of these methods, namely, immunization with the living attenuated E strain typhus vaccine, is being applied on a pilot, investigational scale in two contrasting epidemiologic situations, one epidemic and one endemic.

THE NATURAL HISTORY OF TYPHUS INFECTIONS: A SIMPLIFIED CONCEPTUAL MODEL

The particular epidemiologic pattern assumed by typhus in any given situation depends largely upon the interactions among the major variables in the system which are recognized as (a) the agent, *Rickettsia prowazeki* (4,5), (b) the vector, the body louse *Pediculus humanus humanus* L. (3), and (c) Man, who serves as host to both agent and vector, as a reservoir of the rickettsia and as a mobile agent whose behavior markedly influences the other variables and determines the particular pattern of transmission (2, 6-8). Interaction is stressed because in large, complex populations, it is possible for vector, agent in its reservoir and susceptible people to co-exist without interacting, as in the U.S.A. today.

In this brief discussion, we will not consider possible variations in properties of agent or vector, only quantitative variations in prevalence; nor can we dwell upon environmental factors, except as they interfere with the application of control measures. We will also ignore the possibility of extra-human reservoirs (17) which, if they do exist, have not yet been demonstrated to play any role in the transmission of disease to man.

Recognizing these restrictions, and ignoring for simplicity many other extremely interesting variables, it is possible to portray the major interactions diagrammatically, as in Figure 1, and to identify empirically several major and some minor epidemiological stages in the natural history of typhus which have both practical and theoretical value. Although other special situations are possible, most naturally-occurring typhus can be fitted into this scheme; the major factors leading to change in stage can be identified and the consequences of the application of specific control measures can be predicted. From the stand-point of typhus control, which is the main topic of this report, the two most vulnerable points of attack at any stage are clearly recognizable as (a) the vector, through vector control measures, and (b) the susceptible human population, through active immunization. This diagram also stresses the crucial role of the reservoir, i.e., the human typhus convalescent who later develops recrudescent (Brill-Zinsser) disease and the long-range consequences of permitting any transmission of agent to occur. We have no proven means yet of

NATURAL HISTORY OF "EPIDEMIC" OR "LOUSE-BORNE" TYPHUS

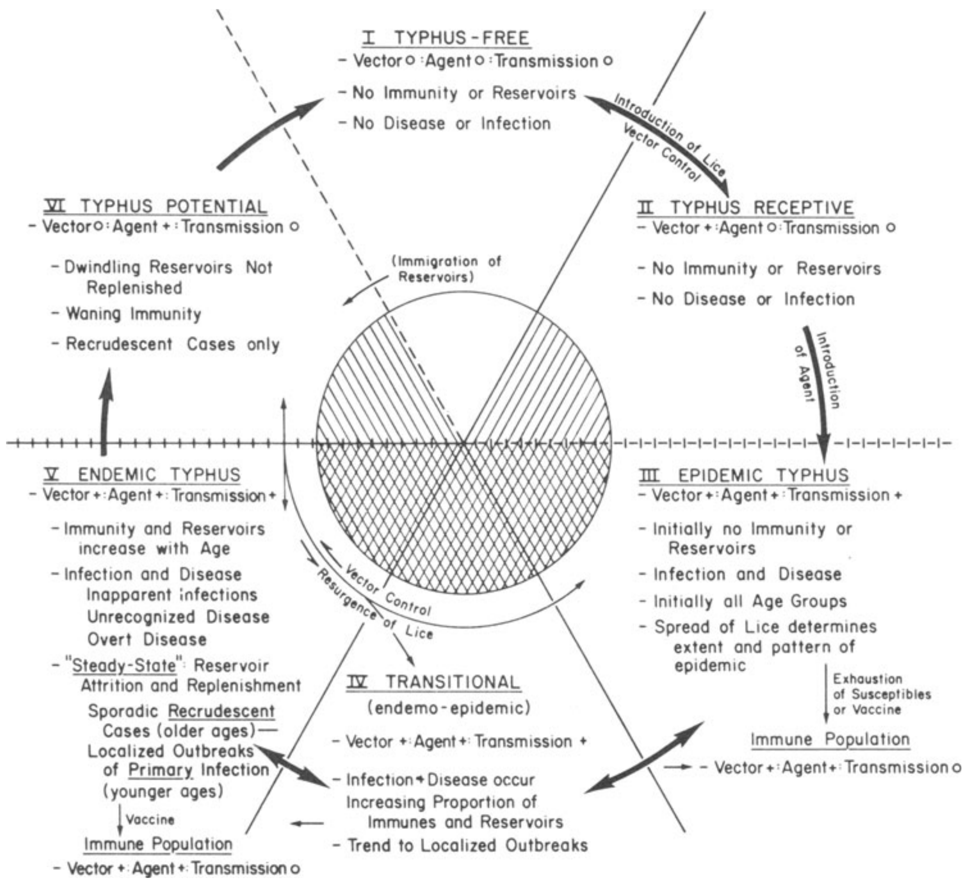


Fig. 1 Diagrammatic representation of the major interactions among the typhus rickettsia, the body louse and human beings in the natural history of louse-borne epidemic typhus fever. Major epidemiological stages are identified by Roman numerals.

eradicating the organism from the human reservoir, where its presence is the key to endemicity; it now cannot be removed except by natural attrition.

Actual examples of all these different stages with complete, fully documented, quantitative data on all the important variables just do not exist. However, the recent history of typhus in Poland, compiled from various sources (9-11) and synthesized into a graphic summary in Figure 2, illustrates many important points. Thus, following the large epidemic during and after World War I, the disease appeared to settle down into an endemo-epidemic pattern, somewhere between Stages IV and V, with a large fraction of the disease follwoing the typical seasonal pattern of louse-borne primary infection, as shown for the years 1936-1939. These cases probably occurred only in certain segments of this large, complex population. No doubt socio-economic compartmentalization of different sub-populations within the whole permitted the preservation of typhus in certain segments side by side with the simultaneous accumulation of large numbers of susceptibles in typhus-free segments. With the disastrous disruptions of World War II, the diaphanous barriers between typhus-bearing and typhus-free segments disintegrated, conditions for widespread lousiness were created in all segments of the population and both vector and agent spread rapidly to produce a massive epidemic, more or less equivalent to Stage III. With the cessation of the war and the return of the population towards a higher standard of living and hygiene, probably aided by the newly discovered insecticides, the incidence of disease diminished and for a time appeared to be settling back into its pre-war endemo-epidemic pattern. In the early 1950's, however, the seasonal variation became less pronounced and the cases were found to consist of two types: (a) recrudescent cases occurring steadily throughout the year in the older age groups who had experienced primary infection years before, and (b) louse-borne primary typhus cases classically in the cold months in the younger age groups who represented the major accumulation of susceptibles. The pattern of typhus had thus shifted into a Stage V or endemic form. Further reduction of the vector with elimination of transmission could easily shift the pattern into Stage VI.

Figure 3 summarizes the age relationship in the endemic situation between immunes, reservoirs and recrudescent typhus cases on the one hand and the susceptibles and primary typhus on the other, which is the mechanism whereby the attrition of older reservoirs through death is offset by the creation of new reservoirs. The figure also shows how either vector control or mass immunization can interfere with this process.

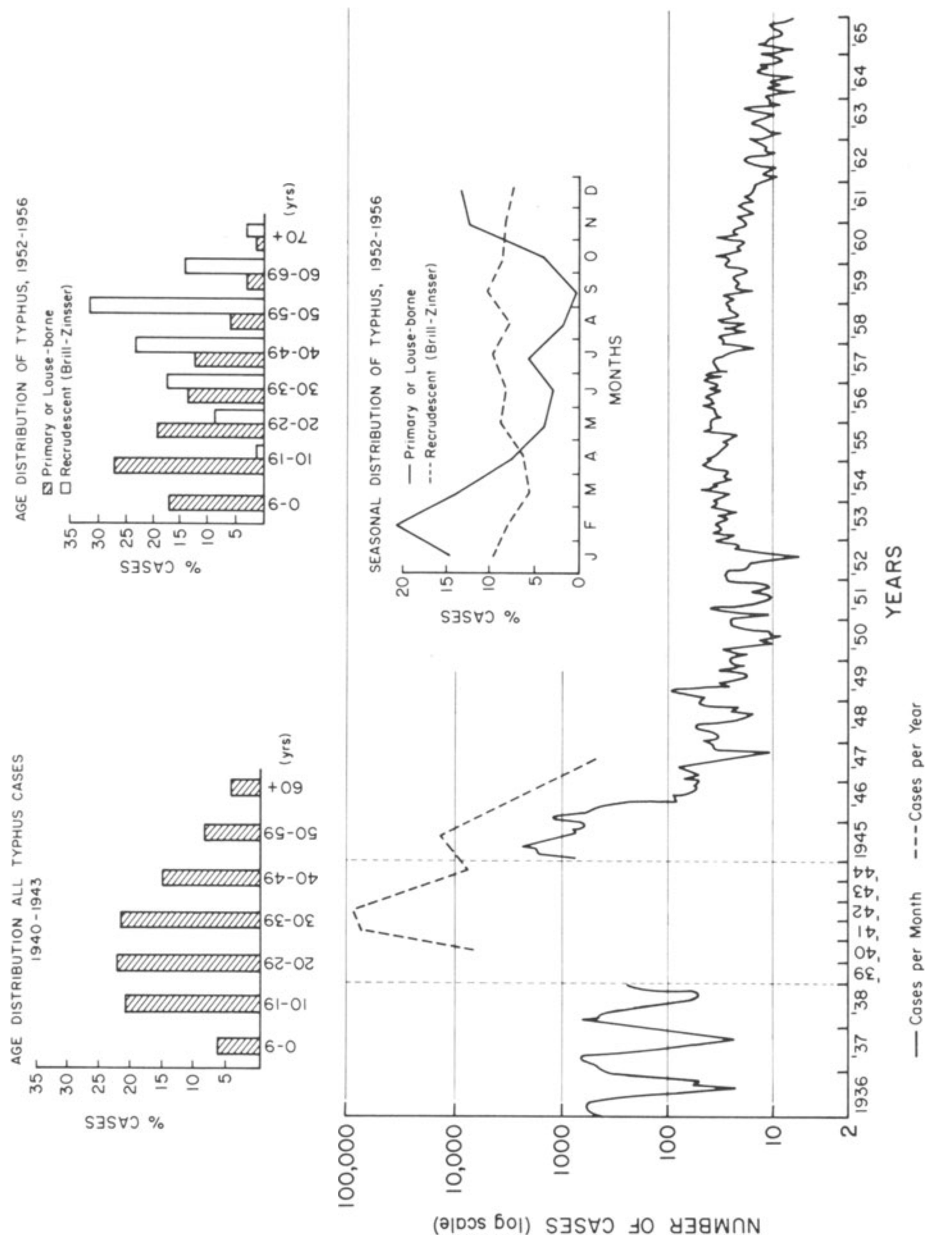


Fig. 2 Natural history of typhus fever in Poland, 1936-1965. Note the shifts from endemo-epidemic to epidemic to endemic with changes in living conditions. (Adapted from data primarily from References 10 and 11.)

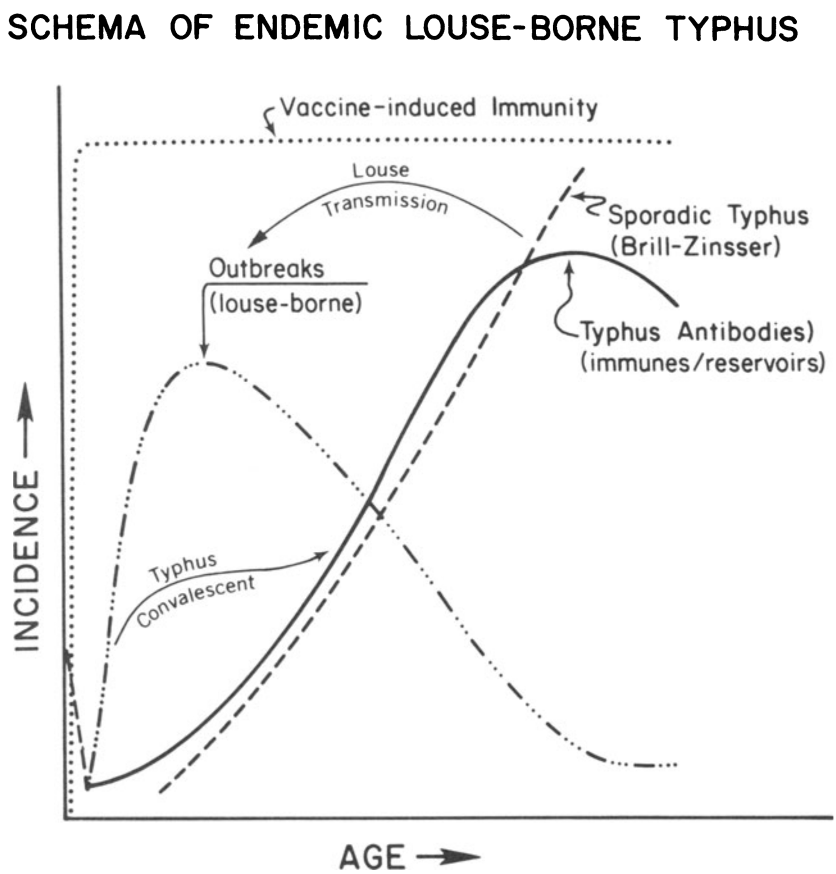


Fig. 3 Schematic representation of endemic state of typhus showing the age distribution (a) of typhus immunes (who are also typhus reservoirs) as revealed by sero-epidemiological studies, (b) of cases of recrudescent typhus derived from "(a)" and (c) of cases of primary louse-borne typhus which replenish the reservoir population which is being depleted by natural attrition, yielding a "steady state" type of equilibrium. Elimination of either the vector or the susceptible segment of the population will alter the dynamics, but in significantly different ways. (Basic patterns of antibody and recrudescent and primary typhus derived from examination of data in References 10-15.)

PRINCIPLES OF TYPHUS CONTROL

In 1937, a Committee of Experts at a conference held under the auspices of the League of Nations (18) summarized the principles of typhus control, based upon the accumulated knowledge and experience at that time (Table 1). In drawing up this list of principles, it would seem that the Committee had in mind primarily the sharp outbreak or epidemic form of the disease typical of the European setting. With minor modifications in details, these principles are still valid for control of the localized outbreak or epidemic. However, additional modifications now seem desirable to define a comprehensive program generally applicable to all epidemiological stages in the natural history of typhus, to large national or regional problems as well as to more localized outbreaks, and to accommodate great variations in logistic aspects and resources. A comprehensive approach must not only be concerned with the urgent immediate problem of disease prevention in any given outbreak but also with the ultimate long-range management of the basic underlying problems if any real impact is to be made on the disease. Table 2 emphasizes the key role that preventing the creation of new reservoirs plays in attaining the ideal or some reasonably acceptable compromise.

Table 3 summarizes some characteristics of the measures currently available for typhus control. It is obvious that none is perfect, but each can be used to advantage under certain conditions.

REALITIES OF THE PROBLEMS OF TYPHUS CONTROL IN DEVELOPING COUNTRIES

In many developing countries, typhus appears to exist in the Stage V of endemicity, if one can rely upon the history, reported cases and scant serological data (19-29). Figure 4, an example taken from the data of Montoya *et al* (19), shows the age distribution of typhus antibodies in 1954 in certain populations in Peru where typhus has been a problem for an untold number of years. It is easy to predict that the pattern of recrudescent and primary cases of typhus, if identified, would conform to that depicted in the preceding Figure 3.

The validity of such an extrapolation from sero-epidemiological data is reinforced by the occurrence of sporadic explosive outbreaks of typhus so common in remote villages of the mountainous regions of the world, such as in Mexico, Central America and South America where there is reason to believe that the sero-epidemiological profile is similar to that just described for Peru (23-29).

TABLE I

GENERAL MEASURES FOR COMBATTING TYPHUS

1937 LON Principles for Epidemic Control	1972 Comprehensive Program
1. Case finding	I. Recognition and Definition of Problem
	A. Reporting and laboratory diagnosis
	B. Epidemiological study and classification of problem
	C. Assessment of logistics and resources
	D. Selection of appropriate control measures from "B" and "C"
2. Isolation of cases	II. Interruption of Transmission
	A. Short-term: Immediate effect
3. Isolation of districts and regions	1. Isolation of cases and regions
	2. Vector control: Insecticides
	a. assessment of resistance
4. Delousing	b. delousing
	(1) Contacts
5. Immunization of individuals and selected groups	(2) Mass program
	3. Immunization: Elimination of susceptibles
a. Vaccine	4. ? Chemoprophylaxis (limited usefullness)
	B. Long-range: Ultimate eradication
b. Seroprophylaxis	1. Prevention of new reservoir formation
	a. Eradication of vector
	(1) ? Insecticides
	(2) Laundry, bathing, education
	b. Maintenance of high state of immunity in all age groups
	2. Elimination of existing reservoirs
	a. Natural attrition
	b. ? Chemotherapy - no rickett-sia-cidal agent yet available
	III. Treatment of cases
	A. Reduction of morbidity and mortality
	B. Reduction of period of infectivity for louse
	C. Prevention of reservoir state (not yet possible)

TABLE II

OBJECTIVES OF TYPHUS CONTROL MEASURES

1. Immediate: Stop disease
2. Intermediate: Prevent creation of new reservoirs of agent
3. Long-range Ideal: Eradication of agent and vector

TABLE III

CHARACTERISTICS OF AVAILABLE TYPHUS CONTROL MEASURES

1. Louse Control
    a. Prevents disease
    b. Interrupts transmission, even if lice are not eradicated
    c. Eradication difficult; effect often transient; resistance may interfere
    d. Prevents creation of new typhus reservoirs
    e. Uninfected population remains receptive to re-introduction of agent
2. Immunization (ideal)
    a. Prevents disease
    b. Interrupts transmission
    c. Prevents creation of new reservoirs
    d. Population unreceptive to re-introduction of agent even if vector is not eliminated
3. Chemotherapy-Chemoprophylaxis (present status)
    a. Probably can prevent disease, but not infection
    b. Shortens period of transmissibility to lice
    c. Probably does not prevent creation of new reservoirs
    d. Reduces morbidity and mortality of established cases

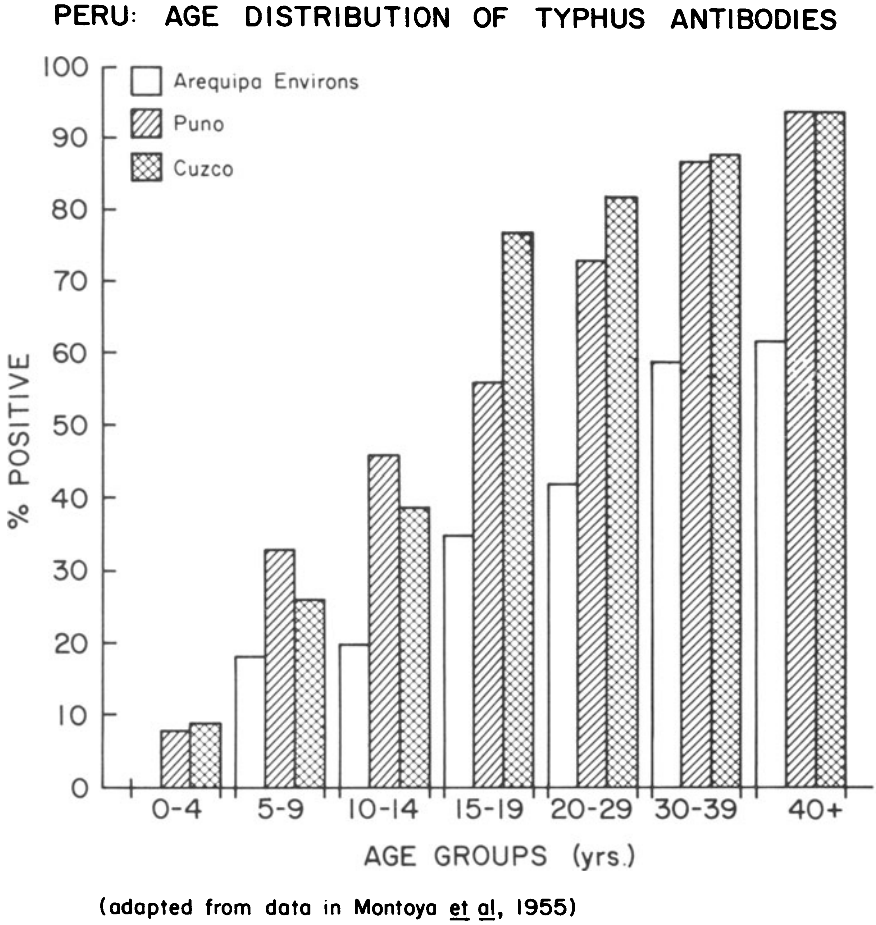


Fig. 4 Age distribution for typhus antibodies in three typhus-endemic localities in Peru in 1954 (adapted from data in Reference 19). Considered as reservoirs, it would be predicted that these typhus convalescents should give rise to a substantial number of recrudescences followed by louse-borne primary infections. Very limited data on age distribution of reported typhus (27) suggest that this does occur but there has been no differentiation between recrudescent and primary cases. Moreover, there is serologic evidence from the same region (64) for the occurrence of between 13 and 51 infections for every recognized case over a 3 1/2 year period of surveillance.

MEXICO: CONTROL OF TYPHUS EPIDEMIC IN A VILLAGE WITH DDT DUSTING

(adapted from Ortiz-Mariotte et al, 1945)

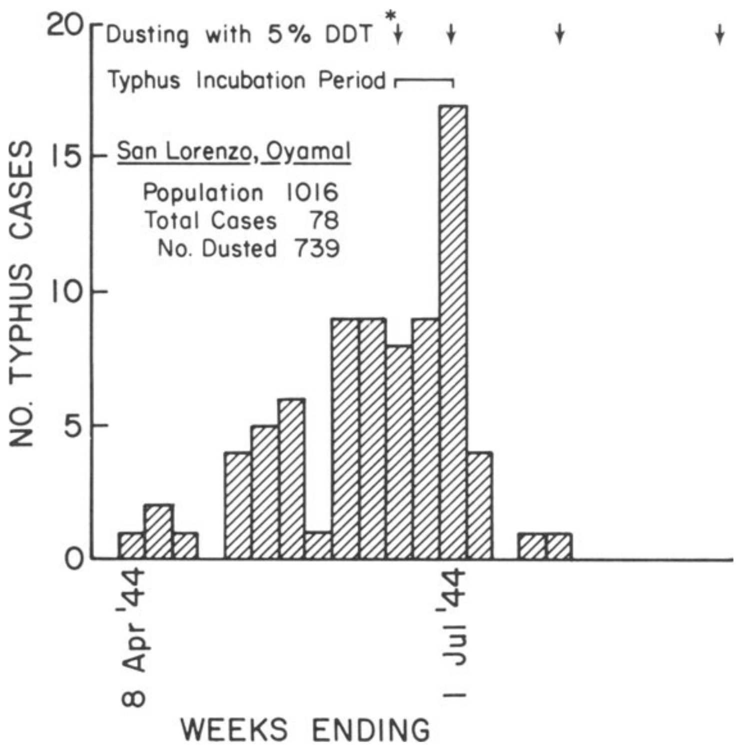


Fig. 5 A typical village outbreak of typhus in an endemic region. Had this outbreak not been controlled with DDT, it would have been expected to follow a natural course typical of village outbreaks in this region in which the declining phase resembles the build-up roughly in shape and time scale. (Adapted from Reference 25.)

The classical story of recognized typhus, repeated so many times over the years in different places where the disease is endemic, is that word is received by health authorities of a local outbreak of serious disease with some deaths which resembles typhus, often in some remote place. A health team is sent out to investigate and control the outbreak. By the time the team reaches the site, after the usual delays imposed by problems of travel, etc., additional cases have occurred. Patients with disease are treated and the population is dusted with insecticides one or more times over a short period, often without knowing whether or not the lice in that locality are indeed susceptible to the insecticide(s) being used. Perhaps even some killed vaccine is administered. The outbreak ceases, as a result either of the control measures or of the seasonal pattern.

However, there is growing evidence that many more typhus infections actually occur in these areas than are recognized and reported (9,64). The sporadic case of recrudescent or primary typhus, occurring on a background of other similar febrile illnesses, is not recognized and inapparent infections also seem to occur. The typical localized outbreak just described is then merely the tip of the iceberg of typhus that is visible while the bulk of the transmission and infection remains unrecognized. Thus, the practice of limiting the attack on typhus to these sporadic outbreaks of overt disease as they occur has in fact several serious disadvantages: (a) it does not recognize most typhus infections which are occurring; (b) it recognizes typhus only when many people have become seriously ill and some have died; (c) new typhus reservoirs have been created; (d) the vector is not eradicated and indeed insecticide resistance may be enhanced; and (e) no permanent or significant change has been made in the basic epidemiological climate or future typhus potential.

Some of the main factors which contribute to typhus endemicity and interfere with optimal application of control measures in developing countries fall into the following categories:

1. Living conditions which are conducive to chronic, endemic lousiness, possibly enhanced by cultural factors related to lousiness.

2. Difficult communications, both in regard to notifying health authorities about disease occurrence and accessibility of population for prompt and repeated application of control measures.

3. Limited resources: economic, trained man-power, transport, etc.

4. Poorly developed systems for reporting disease and inadequate laboratory diagnostic facilities.

5. Political: hostility of population segments towards representatives of central government; rejection of government-sponsored programs (30).

6. Control measures and concepts based on experience with unusual acute, transient situations in advanced countries are applied directly, and sometimes inappropriately, to the chronic, persisting conditions in the less well-developed, emergent countries.

Though not exhaustive, this list identifies some of the factors which may influence the choice and efficacy of control measures in any given situation.

APPLICATION OF SPECIFIC CONTROL MEASURES TO TYPHUS PROBLEMS IN DEVELOPING COUNTRIES

Vector Control Through Application of Insecticides

Because of the early dramatic successes in the control of typhus by the mass application of insecticides in Europe during and immediately after World War II (25-28), great reliance has been placed on this measure for control of typhus (35). However, this approach has not always met with such success in some of the less well-developed areas of the world for the reason that inadequate attention has been paid to the basic epidemiological, ecological and biological factors, to the enormous logistic problems and to the limitations in resources.

In retrospect, the situations in which insecticides have had their greatest effect on the long-term control of typhus differed significantly from the conditions existing in most areas with current persisting typhus problems. For example, the dramatic effect on the 1943-44 typhus epidemic in Naples was probably not only due to the powerful action of DDT on the louse (33) but also to the fact that the conditions contributing to lousiness were transient; summer was approaching and there was a relatively rapid return towards the former higher standard of living and hygiene, which then held lousiness at a reduced level without continued intense insecticide application. Moreover, DDT dusting was accomplished efficiently and rapidly through the enormous logistic potential and resources of an organized military force.

In sharp contrast, the conditions conducive to lousiness, and lousiness itself, in less well-developed countries where typhus is a problem, are not transient, but tend to be long-standing, persistent and chronically endemic - almost a way of life. When an insecticide is applied once, or perhaps even a few times over a short period of time in this setting, there may be a prompt initial, but evanescent, reduction in lousiness (27, 31-34); but after a short time the incidence of lousiness may again approach its former level (27), as shown in Figure 6. Thus, to achieve long-term vector control without basic changes in the way of life, a vigorous program of indefinite duration, consisting of repeated insecticide application at appropriate intervals, must be maintained, unless complete eradication can be achieved without subsequent re-infestation. This may be logistically impractical or even biologically unsound.

Despite these limitations, insecticides have had dramatic effects on the small localized outbreaks of typhus which characterize the endemic or endemo-epidemic situations, as shown in Figure 7. Thus, a single or a few applications of insecticide over a short period of time can reduce lousiness to a sufficiently low level that the transmission chain of typhus is broken and the outbreak ceases, even though the louse population may subsequently return to its former level and the basic epidemiological state is unchanged.

However, when insecticides are used suboptimally in the face of endemic lousiness, the lice may develop resistance to the insecticide (35). Figure 8 illustrates another localized explosive typhus outbreak in a Mexican village, typical of the endemic situation, about two decades after the initial introduction of DDT into Mexico; a little over a decade after institution of a nation-wide campaign to eradicate the body louse; and about five years after DDT-resistant lice were first recognized in Mexico. In contrast to the sharp effect of DDT in the previously described outbreak, there is really no discernible effect of mass dusting with DDT in this instance. The disease continued to follow the expected seasonal pattern of an uncontrolled outbreak in this setting. Although data are not available for this village, one can only assume that the lice had become resistant to DDT.

Today DDT-resistance is common among body lice in many parts of the world, resistance to lindane (gamma-hexachlorohexane) is being reported with increasing frequency (35), and a high degree of resistance to malathion has been found in Burundi (36).

These are a few examples of the problems being encountered in attempts to control the typhus vector with insecticides in endemic situations. Other approaches to louse control, too extensive to consider here, are possible and are probably feasible under these circumstances.

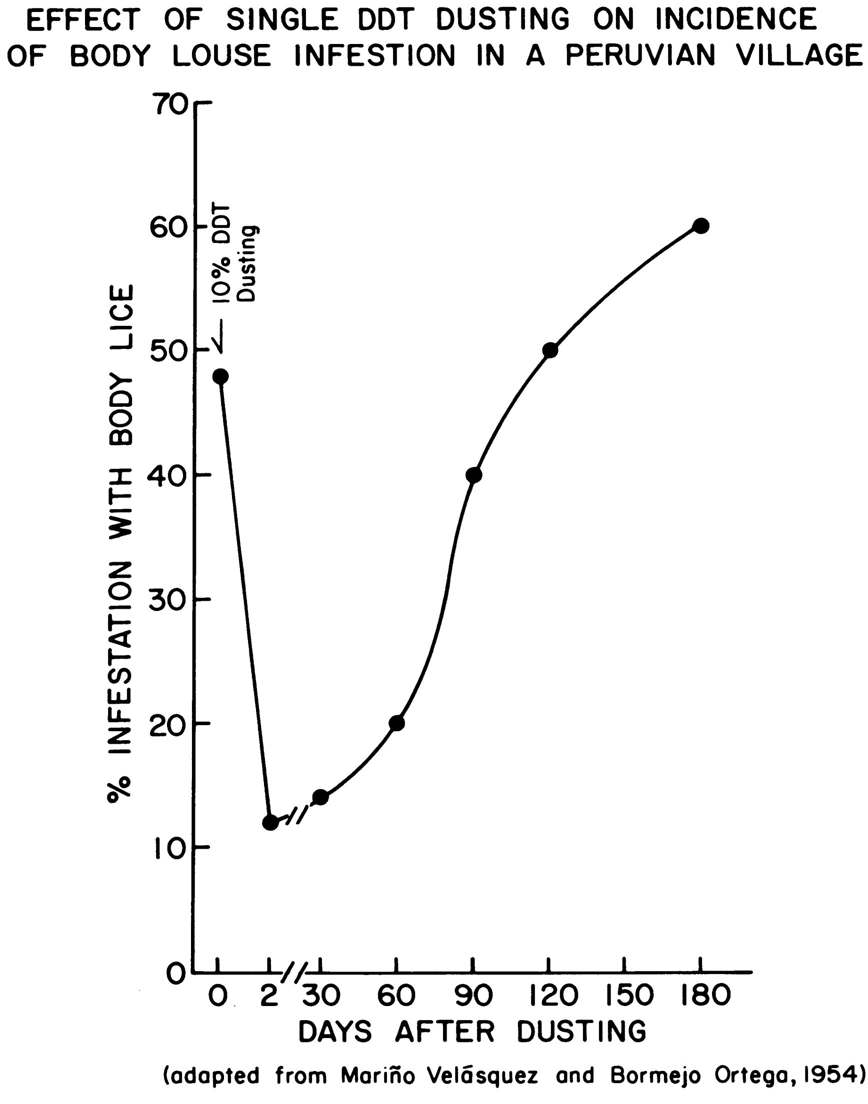


Fig. 6 Effect of a single DDT dusting on the incidence of lousiness in a Peruvian village in a typhus-endemic zone (adapted from Reference 27). Note the rapid and dramatic - but not complete - reduction in the incidence of lousiness after dusting, followed by the gradual rise. Quantitative data of this kind collected over such a long period of time are rare in the literature. Though inadequate to eradicate lice, an effect of this kind may interrupt the transmission cycle of typhus in typical village outbreaks.

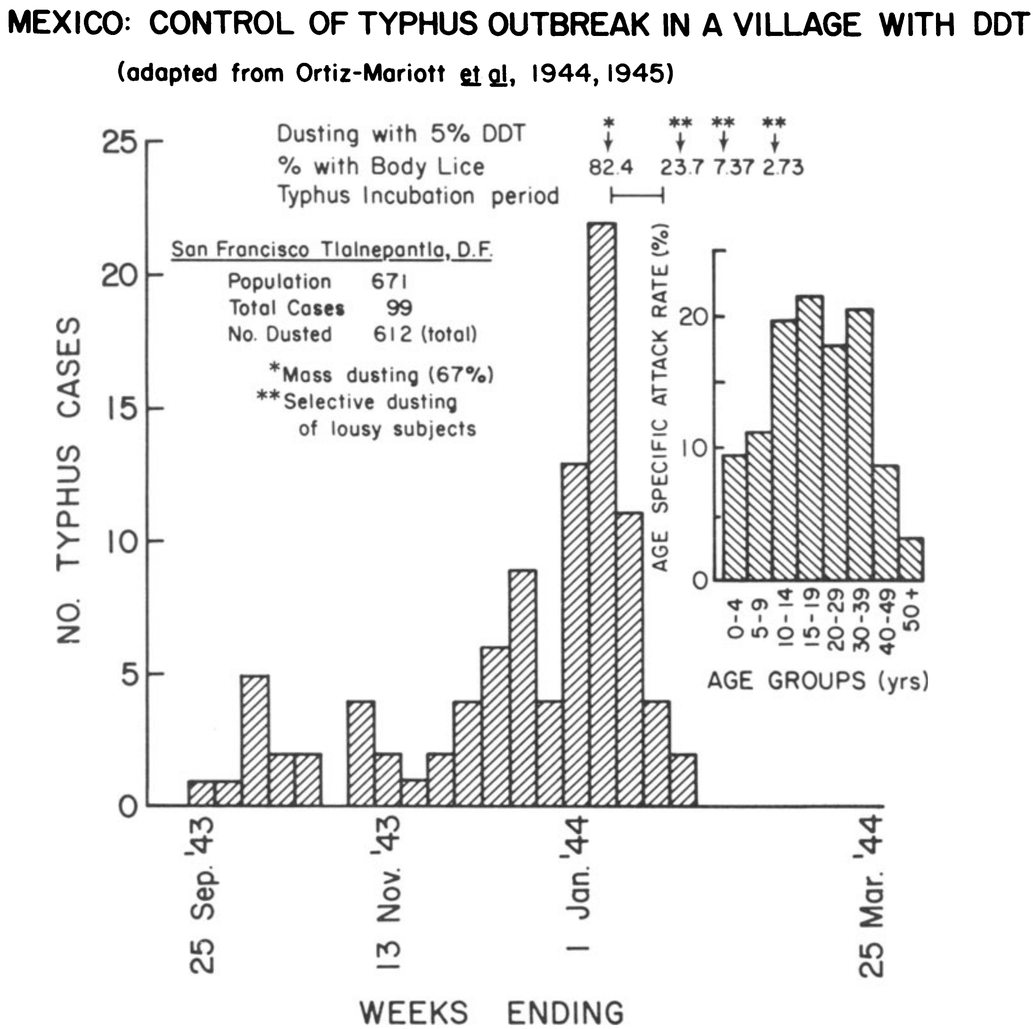
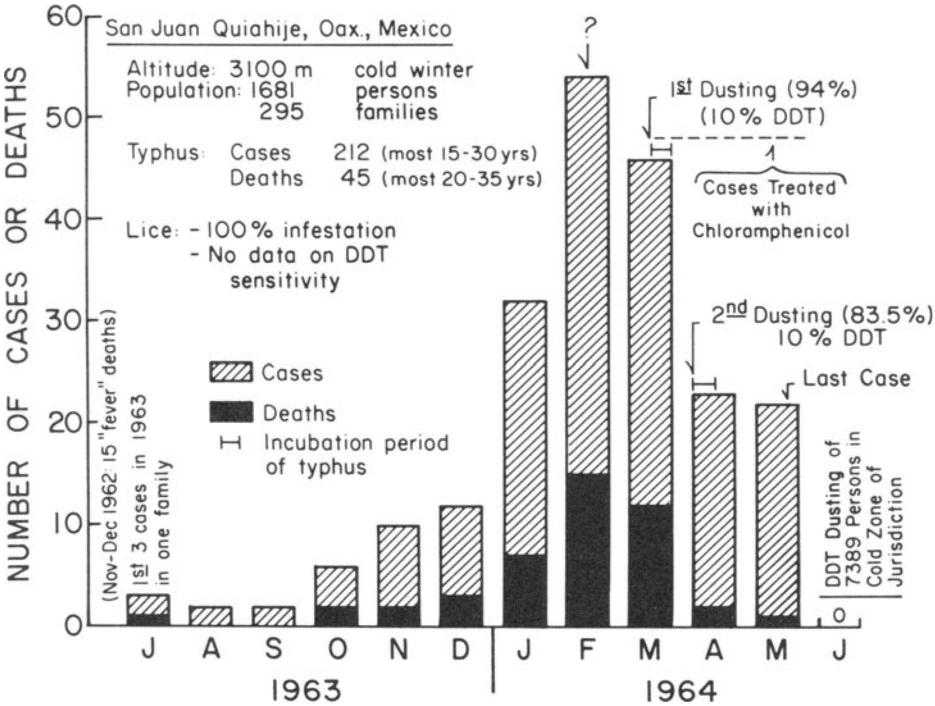


Fig. 7 Control of a typical village typhus outbreak in an endemic zone with repeated applications of DDT (adapted and interpreted from data in References 25 and 26). Note the typical age distribution of cases and the significant - but not complete - reduction in lousiness, which would be expected to rise again, as demonstrated in Figure 6. In the outbreaks illustrated here and in Figure 5, there was a marked reduction in incidence of typhus cases within one incubation period of typhus after the first application of DDT.

WINTER TYPHUS OUTBREAK IN MEXICAN MOUNTAIN VILLAGE: POOR RESPONSE TO MASS DDT DUSTING-? UNRECOGNIZED RESISTANCE.* (Compiled from Olivero Toro and Ortiz Mariotte, 1964)



*DDT resistance in body lice first reported from Mexico in 1959 (WHO)

Fig. 8 Failure of DDT to alter the course of a village typhus outbreak, probably because of unrecognized DDT-resistance among body lice. Note that (a) the time increments here are months, instead of weeks as in Figures 5 and 7, (b) there was little recognizable effect of dusting within an average typhus incubation period and (c) the course of this outbreak is very similar to that of untreated village outbreaks. (Assembled, adapted and re-interpreted from Reference 23).

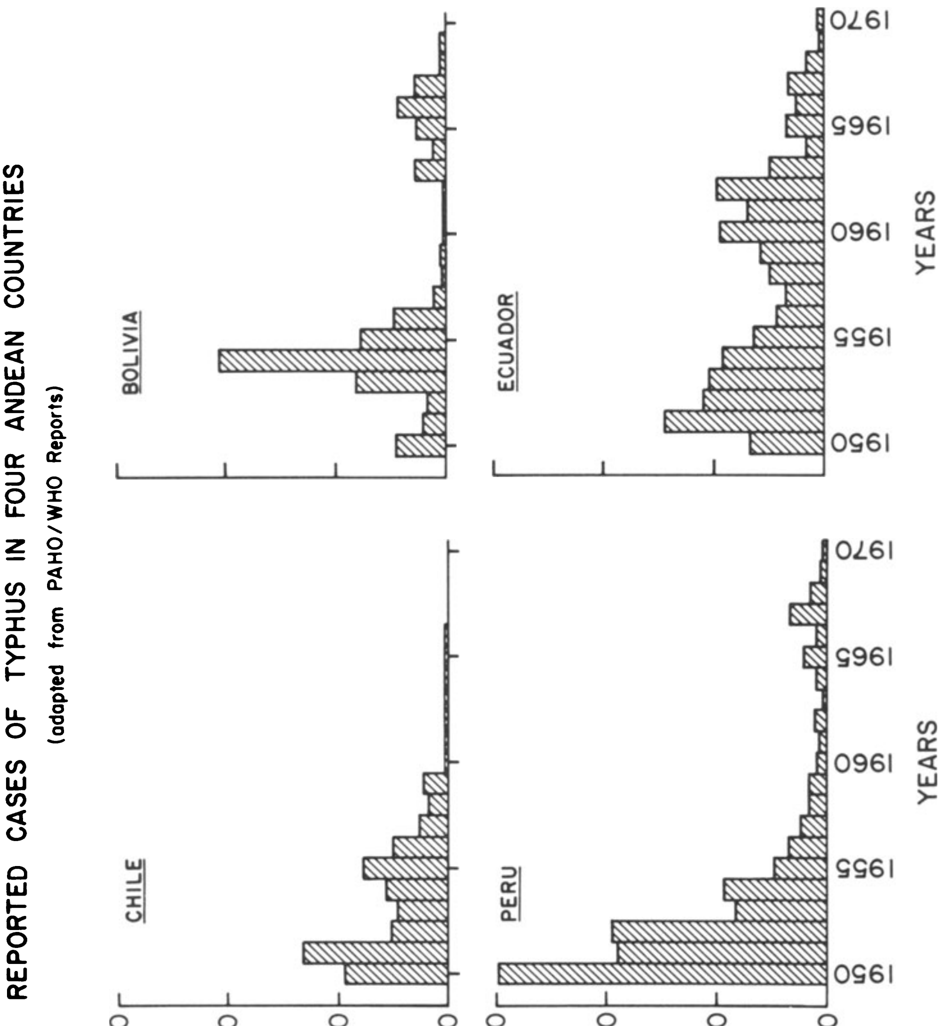


Fig. 9 Reported cases of typhus (1950-1970) in four Andean countries where typhus has been endemic.

Despite the gloomy picture presented above, louse control through insecticides is an important component of typhus control and when used appropriately can accomplish: (a) a transient effect sufficient to stop small localized outbreaks of disease, even though the vector is not eradicated and conditions conducive to lousiness persist; (b) eradication of lice in small populations not subject to re-infestation; and (c) acceleration of the disappearance of lice when basic living conditions are improving. However, it is questionable if insecticides alone can eradicate or even produce long-term control in the vast, relatively inaccessible populations of depressed areas where conditions conducive to lousiness remain unchanged. Nevertheless. there has in fact been a gradual decline in reported cases of typhus from several countries which have instituted some kind of louse-control program (27-29), as is illustrated in Figure 9. The explanations for this, however, may be complex, including a possible rise in standard of living, health education and other factors, and may not be entirely the result of the insecticide programs.

Elimination of the Typhus-Susceptible Segment of the Human Population Through Active Immunization

Immunity following typhus fever is usually strong and long-lasting; naturally occurring antigenic shifts in *R. prowazeki* have not been proven. Since even the immunity following natural typhus fever is non-sterile, i.e., the organism persists, it is unknown if even the best possible vaccine would prevent a vaccinated person, on subsequent exposure to a virulent strain, from acquiring and harboring that strain, thus becoming a potential reservoir. At the opposite extreme, neither is it known if vaccine given to typhus convalescents, i.e., reservoirs, would prevent recrudescence of infection. Nevertheless, immunization with a good vaccine could be expected: (a) to prevent disease in the individual; (b) to prevent transmission, and hence reservoir creation, in the immunized population even if the louse persists; and (c) to protect lousy typhus-receptive populations against introduction of the agent. Immunization of the majority of a population would thus divert it from subjection to the usual epidemiological sequence and into a special category. And if a high level of immunity were maintained for a sufficiently long period of time, natural attrition of reservoirs could proceed in the presence of lice without the risk of outbreaks of primary infection.

Realization of these ideal objectives depends upon (a) the capacity to produce an acceptable safe vaccine which will induce the desired degree and duration of immunity and (b) the feasibility of administering it to a population according to the regimen required for effectiveness.

Variations of two general types of typhus vaccine, (a) killed and (b) living, were explored from the earliest days (2,37). For the most part, however, yolk sac-grown, killed vaccines were used by the American, Canadian and British Forces in World War II (38) and have been used by many countries for both civilians and the military since then. These vaccines, unfortunately, are still subject to many problems of production, antigen content, potency and assay, recently reviewed in other reports (39,40). Aside from very limited vaccination-challenge studies, which suggested that some, but not complete, protection was afforded by certain vaccines (41, 42,57,59), no large-scale adequately controlled trials have been carried out successfully so as to provide the necessary quantitative data on effectiveness. Moreover, the combined experience of the Allied military forces with killed typhus vaccines in World War II did not yield satisfactory information on the degree of protection afforded against acquiring typhus fever even though it clearly indicated that the vaccines modified the disease and prevented deaths in persons who developed typhus after having received one or more doses of vaccine (43-47,65,66). In addition, the infectivity for lice of patients with vaccine-modified disease was found to be reduced but not eliminated (48-51). These considerations, along with the requirement for multiple doses and frequent boosters, suggest that currently available killed vaccines may not be adequate to achieve the potential benefits of immunization mentioned above, especially with regard to changing the basic epidemiologic status.

With the recognition of a spontaneous attenuated mutant of *R. prowazeki*, the Madrid E strain, by Clavero and Perez Gallardo (52,53), a new candidate for a living attenuated vaccine was introduced. Fox and his associates (54-64) explored its potential as a vaccine in a series of comprehensive studies from the laboratory, through vaccination-challenge studies in human volunteers, to a large-scale field trial in Peru in the 1950's. Most significantly, from the standpoint of typhus control programs, as we have presented the problem here, was the fact that a single inoculation of the living attenuated E strain produced a strong immunity to virulent epidemic typhus challenge which lasted for at least five years. Two types of reaction to the vaccine were encountered: (a) a dose-dependent early reaction (24-48 hrs) which could be eliminated by adjustment of dosage and (b) a late reaction in up to 14 percent of the people, 9-14 days after vaccination, which varied from mild malaise and headache to a brief highly modified febrile illness which never required treatment. These reactions, however, did not interfere with the acceptability of the vaccine in the field trial in Peru which involved over 30,000 people. Other groups have since investigated the E strain to greater or lesser degrees (65-78), with somewhat variable conclusions regarding the importance of reactions as regards acceptability (see next section).

TABLE IV

COMPARISON OF KILLED AND LIVING ATTENUATED TYPHUS VACCINES

Category	Characteristic of	
	Killed Vaccines	E Strain Vaccine
1. No. of Doses	Multiple; timing important	1
2. Time for Effect	Relatively long for optimum	Prob. 7-10 days
3. Duration of Immunity	? Short, requiring periodic boosters. Good booster after many years	$\geq$ 5 yrs
4. Protection		
a. Clinical Disease	Incomplete but modifies	Strong
b. Rickettsemia	Incomplete but reduces	Prob. none
c. Louse Infection	Incomplete but reduces	Prob. none
d. Reservoir Creation	Incomplete prevention	Unknown
e. Recrudescence		Unknown
5. Reactions	1) Local and systemic "endotoxin" reactions with potent vaccines	1) Early dose dependent in both immunes and non-immunes
	2) Local and systemic delayed type hypersensitivity	2) Late reactions--modified disease in non-immunes
	3) ? Immediate allergy to host cell components	
6. Problems	1) Protective antigen(s) not known	? Reversion to virulence
	2) Potency and potency assay	

The E strain has been under study at the University of Maryland for well over a decade and a comprehensive account of these studies is being prepared for publication (79). Experience with its use as a vaccine is being accumulated in a step-wise manner in military populations in the U.S.A. and in different epidemiologic situations with regard to typhus control in developing countries. Although these studies are still in progress and observations are still being made, some of the results available to date, summarized very briefly below, would appear to have some bearing on the problems of typhus control in developing countries.

The first study to be described is a controlled field trial of the E strain vaccine during a large-scale typhus epidemic in Burundi. The sequence of events leading to the occurrence of typhus on an epidemic scale, essentially equivalent to Stage III, in Burundi is complex and is being documented and analyzed elsewhere (36,80, 81). Suffice it to say here that a very large susceptible population, including all age groups, had built up throughout the country, that body lice had become highly resistant to DDT over much of the country and to malathion over extensive but lesser areas and that lousiness was essentially universal throughout the highland regions. A major spread of typhus began in the early 1960's and rose to a very high level of epidemic activity in many parts of the country by 1967-1969, when our studies were initiated. The dispersion of the indigenous population over the land probably influenced the rate and pattern of spread. At the present time, the disease is probably entering the transitional or endemo-epidemic Stage IV of typhus in parts of the country. For all of the reasons previously mentioned, it was not possible for government health authorities to mount an effective louse control program for the entire country, although some success was achieved with lindane-containing powders in isolated instances of accessible populations concentrated in refugee camps.

After identifying the epidemic as louse-borne typhus in 1967 and making some preliminary observations on its epidemiology, a controlled field trial of the attenuated living E strain vaccine was proposed, accepted, planned and finally initiated in 1969. Ten hills in the communes of Katara and Matongo, determined by inspection to be one of the most densely populated and active typhus areas at that time, were selected for the project.

Although the local conditions might impress the casual observer as being too primitive to permit controlled study, closer observation would reveal that all the components for a sophisticated study were in fact present: (a) a large epidemic of typhus in progress; (b) a stable, dense (500-600/sq. mile) population, extraordinarily sophisticated in the recognition of the various clinical types of typhus, who feared the disease, greatly desired help, were extremely cooperative, and who could be identified through the tax-book required to be carried by the head of every household; (c) a sophisticated local civil organization with interested and conscientious local administrators at the commune and colline level, who participated actively and enthusiastically in all phases of the project and whose help with local customs was invaluable; (d) an excellent local communication system, with roads to each colline and drums to spread the word; and (e) a single medical facility, a mission hospital-dispensary, where all illnesses from the region received treatment. Indeed, it would be difficult to find a better-suited local organization and situation anywhere for a study of this kind.

In June and July 1969, 11,164 persons above about one year of age (about 80 percent of the population estimated from census data) were vaccinated by jet gun with a single dose of either Tetanus-Diphtheria toxoid (5,590 persons) or $10^{5.5}EID_{50}$ of the E Strain Vaccine (5,574 persons). People from each hill were directed into the line for one or the other vaccine as they came. A punch-card bearing pertinent data was prepared for each vaccinee. The two groups were remarkably comparable with regard to age, sex distribution and history of previous typhus. The centrally located Katara Mission Dispensary served as headquarters for the study and was the only medical facility in the region where treatment could be received. Numbered study identification cards of the same color as the Dispensary cards were issued to each vaccinee. An intensive surveillance program was instituted for the first 11 weeks following vaccination consisting of a daily ambulance service to each hill to transport <u>any</u> sick person and house-to-house visits (each house was numbered) by health workers and team members every seven to ten days with the help of the local "notables". After the first 11 weeks, when the team from the U.S. left and the pattern had been established with the population for prompt free treatment at the dispensary, modified surveillance was instituted in which all patients presenting at the dispensary were screened and managed, as during the intensive surveillance, by a single, highly interested Belgian Sister who had become extraordinarily competent in this program. Serum specimens were obtained from a sample of the population at the time of vaccination and again six weeks later. Each ill person had at least paired serum specimens drawn, had a detailed clinical record maintained and was given appropriate treatment.

The pre-vaccination distribution of antibodies was comparable in both control and E strain groups. Six weeks after vaccination a marked shift in antibody pattern was found in the E strain group, with only two of 181 individuals in the sample failing to show complement fixing antibody conversion whereas the antibody pattern of the control group was essentially unchanged. Thus, as judged by antibody response, the E strain vaccine accomplished what it theoretically should have done, i.e., changed the basic epidemiological climate and removed the population from the expected natural epidemiological sequence, as shown previously in Figure 1.

Surveillance for typhus cases showed that this was indeed the case as far as prevention of disease is concerned. In the first 11 weeks of intensive surveillance 25 cases of laboratory-confirmed typhus occurred in the control groups whereas none occurred in the E strain group. And at the end of 14 months, which included about 11 months of modified surveillance, 49 cases of confirmed typhus occurred in the control group and three cases in the E strain group, a protection rate of 94 percent. We are now assessing the effect over the second year of modified surveillance.

The E strain vaccine has shown its capacity to protect against disease in the face of an epidemic. If a sufficient proportion of a population were vaccinated, these data suggest that transmission would promptly cease and the creation of new reservoirs would stop, even without a change in the incidence of lousiness. This could have been done throughout Burundi if the vaccine had been given on a mass saturation scale, but vaccination programs of this kind must progress in a step-wise manner to prevent unexpected catastrophes and to gain sufficient experience and confidence. In the meantime, however, an enormous typhus reservoir has been created and the disease, now entering the transitional Stage IV will likely proceed to a stable endemic Stage V which will be difficult to manage by vector control alone, unless there is a very dramatic improvement in the capacity of the country as well as its neighbors to <u>mount</u> and <u>maintain</u> a truly effective country-wide louse control program.

Another field study of the E strain vaccine was initiated last year in Bolivia where typhus has been endemic, probably for centuries. The overt disease pattern here has been one of isolated and localized, sporadic outbreaks of typhus, typical of the highly endemic Stage V. Three widely separated populations, totalling about 8,000 people and representative of the typhus situation on the Bolivian altiplano, were selected for study. Because the efficacy of the E strain vaccine in an epidemic in Burundi in a controlled trial had been demonstrated and because of logistic problems related to resources and accessibility of populations, the plan in Bolivia was (a) to study the epidemiology of typhus in these areas, (b) to vaccinate the total populations in each area, if possible, (c) to assess acceptability insofar as possible, (d) to measure the antibody conversion and persistence patterns and (e) to assess the impact of the vaccine on this endemic situation by whatever means might be possible. This study is still in its early stages. However, it was possible to obtain pre-vaccination serum specimens from over 16 percent of these populations and to vaccinate nearly 60 percent down to about one year of age without incident. Six-week post-vaccination serum specimens have been collected but testing is not yet complete.

The age-specific CF antibody distribution in the pre-vaccination sera from all three areas showed a definite but low incidence in the very young, progressing steadily to an incidence of between 60 and 70 percent of people in the 50-59-year age group. The age distribution of antibodies is typical of the endemic Stage V. Though the data are not available, it is obvious that recrudescent typhus must be occurring in these older people and that louse-born transmission is taking place, often to the young if this degree of endemicity is to be maintained. It is also obvious that, if this pattern is representative of the situation throughout the altiplano, an enormous number of unrecognized infections must be taking place. Only the sharp localized outbreaks involving numbers of people are now recognized, i.e., just the tip of the iceberg previously mentioned. We are hopeful that the E strain vaccine will change the basic epidemiological climate here as well and will prevent the creation of new reservoirs as now must be occurring through inapparent, unrecognized or overt infections. To do so, however, the young must be included in the vaccination program, for these form the bulk of the susceptibles who now maintain the louse-borne transmission phase of the disease. It is of interest, but not significant with regard to assessing the protective action of the E strain vaccine, that a typical sharp outbreak of typhus with 31 cases and seven deaths has been recognized recently in a village near one of our study populations. We hope to be able to continue these studies on a quantitative basis in order to assess the validity of our concepts and the usefulness of the E strain vaccine.

PROBLEMS OF THE E STRAIN VACCINE

From the very beginning of studies on the E strain as a vaccine for man, as with any other living vaccine, investigators have been concerned with: (a) reactions in persons receiving the vaccine, i.e., acceptability; (b) potential for unrestricted growth in the person whose defense mechanisms have been compromised; and (c) stability of the state of attenuation, both in regard to possible increase in virulence which might place both individual and community at risk as well as possible decrease in virulence resulting in loss of immunogenicity. These are discussed separately below.

Reactions to E Strain Vaccine

As with all vaccines and especially with living vaccines, the matter of reactions looms large in importance as regards acceptability and usefulness. Since few vaccines are completely devoid of any reactions whatsoever, the suitability of vaccines are often judged on the basis of the frequency and severity of reactions relative to the need for, and benefits expected from, a given vaccine under a given set of circumstances. For example, smallpox vaccine is not without reactions and even severe risks, but there is no hesitancy to use it when smallpox is a real threat. Indeed, in the past, deliberate inoculation with virulent variola virus was accepted by some as preferable to naturally acquired disease. In many countries now, however, the threat of smallpox is so small that even the risks entailed with the use of vaccinia virus vaccine are considered too great. In the same way, the relative merits of the E strain vaccine should be considered against the typhus disease problem in any given situation.

The unique immunogenicity of the E strain undoubtedly rests upon its capacity to induce an attenuated but immunizing infection in man (54-79). Indeed, administration of chloramphenicol to personse inoculated with E strain suppresses the antibody response (79). However, it has not been possible to isolate the E strain from vaccinated human subjects, either by the inoculation of eggs with blood or by the feeding of lice on such subjects (55,64,82).

On careful observation, two kinds of clinical responses, i.e., reactions, have been recognized following inoculation of human subjects (54-79).

(a) An early reaction, both local and systemic, in the first two to three days after inoculation. This early reaction is dose dependent and can be avoided by reducing the dose, within limits, without significantly depressing its immunogenicity. It occurs in both non-immune and immune subjects.

(b) A late reaction, usually 9-14 days after inoculation and considered by most as an expression of attenuated infection. It is not influenced greatly by dose and is suppressed in immune subjects. Some manifestations of the late reaction have been observed in up to about 14 percent of non-immune subjects. Its severity ranges from simple malaise and mild headache to a modified typhus characterized by fever, headache, malaise and occasionally a rash in a small proportion of subjects. Even the most severe reactions resolve without specific therapy but apparently may be shortened by antibiotics. There is some suggestion that, like typhus itself, these late reactions are more severe in middle-aged persons than in the younger age groups.

The late reaction is the one of concern regarding acceptability of the E strain as a vaccine. Judgements regarding the relative importance of these reactions must be made on the basis of the morbidity it produces under the different conditions of use. Thus, Fox and his co-workers (63,64) found that the E strain vaccine was generally well tolerated in a large field trial in a typhus-endemic area in Peru. Our own studies in the U.S.A. in over 1,000 military recruits in the 18-20-year age group revealed that the E strain was as well tolerated as the killed vaccine, as judged by interference with duty (79). Russian investigators (65-75) found the reactions tolerable in their early experience with the E strain but became somewhat more concerned about them as the numbers of inoculated persons increased. Indeed, they have more recently investigated, with some success, the possibility of giving both killed and living attenuated vaccines simultaneously to suppress the late reactions. Egyptian investigators (76,77) reported that the incidence and severity of reactions were tolerable and did not affect the daily activity of 1,350 persons vaccinated in a village near Cairo. Finally, we have found that the E strain vaccine was acceptable to the populations in our recent field trials in both Burundi and Bolivia. More information of this kind is needed. However, the growing experience is that the E strain vaccine is well tolerated by populations in typhus zones; and the reactions experienced are indeed minor compared with full-blown typhus fever.

Potential for Unrestricted Multiplication in the Person Whose Defense Mechanisms Have Been Compromised

It is possible that an agent which behaves as an attenuated strain in normal healthy subjects might behave as a virulent strain in the subject whose defense mechanisms have been compromised by genetic defect, acquired disease or physical and chemical agents. Only a limited number of animal studies bearing on this possibility have been performed with the E strain. Thus Genig (83), comparing the virulent Breinl and the attenuated E strains in guinea pigs which had been stressed with cortisone, found a definite intensification of infection caused by either large or small doses of the virulent Breinl strain but could demonstrate only slight intensification of infection even with very large doses of the E strain and no effect with small doses. Weiss and Dressler (84) found that heavy irradiation of chick embryo entodermal cells in cell culture did not enhance infection with the E strain, but simply elicited a more rapid release of the microorganisms during the period in which the host cells lysed. Fabrikant (79,85) failed to find evidence for enhanced infection with the E strain in both mice and guinea pigs which had been subjected to very heavy doses of whole body X-irradiation. Thus, the limited evidence available to date does not suggest that the E strain readily exhibits dangerous properties in the host whose defenses have been compromised by these two mechanisms.

Stability of Attenuation

The properties of most microorganisms can be altered by manipulation of the selective pressures to which they are exposed. In the case of living attenuated vaccines, the important question is whether or not important changes in properties, i.e., reversion to virulence or the acquisition of new undesirable properties on the one hand or loss of immunogenic capacity on the other, are likely to take place under the conditions of production and use as a vaccine.

When the information available today about the E strain is examined from this point of view, one finds that its properties are remarkably stable under the conditions of production and use as a vaccine. Thus, the vaccine is produced by most investigators by growth in the yolk sac of embryonated hens' eggs. It is the opinion of all the investigators whose work has been reviewed (54-79,86,87) that serial passage in the yolk sac, which now has attained upwards of 270-500 passages, neither enhances nor diminishes its virulence. Indeed, in this host it has displayed a most remarkable stability of attenuation.

When one examines the information that is available about stability of attenuation under conditions of use as a vaccine in man, one finds similar evidence of stability. Although the evidence presented above suggests that the E strain rarely, if ever, causes sufficient rickettsiemia in man to infect lice, several investigators have studied the behavior of the E strain in human body lice. The E strain has been found to grow in the gut epithelium of artificially inoculated lice, but the degree of involvement of the louse gut epithelium has been reported to be lower and the destructive changes less prominent than in the case of infection with a virulent strain (57,82,88,89). Three different investigators have studied the effect of serial louse passage (1-25 serial passages) on the virulence of the E strain and have found no change in the state of its attenuation (82,89,94). Thus, under conditions of use as a vaccine, it would seem highly unlikely that lice would become infected with the E strain but, even if the rare louse did become infected and transmit the strain, it is not likely that this passage through the louse would be accompanied by an increase in virulence.

One possible situation has not been investigated, although there is no evidence yet for its occurrence. It is conceivable that in the rare instance there could be reversion to virulence within the vaccinated human subject. If this did occur, it would not be likely to cause any immediate threat, either to the vaccinated individual or to the community, because (a) such a reversion would presumably occur only in a very small fraction of the organisms present and (b) immunity induced by the attenuated organism develops so rapidly that the hypothetical virulent mutant would have little chance to multiply sufficiently to cause serious disease in the vaccinated person or to infect lice. The possibility, however, that such hypothetical virulent organisms persisting in the vaccinated subject might constitute a reservoir for future recrudescence cannot categorically be excluded at this time and warrants investigation.

When viewed from the perspective of production and use of the E strain as a vaccine for man, the interesting and important, but not entirely surprising, finding of Balayeva (90-93) that a virulent strain, stable in subsequent egg passage, can be selected from the E strain on serial passages in the lungs of mice loses much of its significance as a deterrent to the use of E strain as a vaccine because in practice such an artificial selective pressure would not occur. Of somewhat greater concern, however, is the report by this same group (91) that virulent strains were isolated after one to a few passages in guinea pigs after heavy inoculation with the E strain. There are certain inconsistencies in this report, viz., the reference to fever persisting as long as five days after E strain - a phenomenon unknown in our laboratory - which make it mandatory that these studies be repeated independently in other laboratories where no virulent strains exist and that additional modern genetic techniques be applied to the study and quantitation of variation in virulence in the E strain.

SUMMARY

A. A simplified conceptual framework of the natural history of typhus, based upon the interactions of agent, vector and human host, has been presented with which it is possible (a) to classify most naturally-occurring typhus through measurements which are readily performed with available methods and (b) to predict the effects of control measures and other factors on the basic epidemiological state.

B. The crucial role of the typhus reservoir, i.e., the typhus convalescent person who still harbors the organism and who may subsequently succumb to recrudescent disease in which the rickettsiae again become accessible to lice, has been stressed as it relates the epidemiology of typhus and to the long-range objectives in typhus control.

C. The special problems related to typhus and typhus control in developing countries -- epidemiological, situational and operational -- have been reviewed briefly.

D. The principles of typhus control and the control measures available have been reviewed, in the context of the epidemiological model of the natural history of typhus infections, with regard to (a) their immediate effects on the occurrence of typhus and their capacity to effect a long-range change in the basic epidemiological climate, (b) their practical applicability to typhus control programs in developing countries and (c) some of their special limitations and problems.

E. The need for, and value of, careful quantitative epidemiological studies of typhus in areas under consideration for control programs are stressed in order to select appropriate control measures and predict their effect on both immediate and long-range aspects of the problem.

F. Preliminary results of field studies in progress on the use of the living attenuated E strain vaccine in both epidemic and endemic typhus in Burundi and Bolivia are described. Practical and theoretical advantages of a typhus vaccine which imparts a durable immunity after a single dose are stressed, especially under conditions where effective louse control programs cannot be mounted and sustained. Both practical and theoretical problems of such a vaccine, i.e., reactions and stability of attenuation, are discussed.

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