STUDIES ON THE INFLUENCE OF INFECTING DOSE ON THE SEVERITY OF DISEASE

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October 1993

ABSTRACT

The influence of inoculum size on severity of disease for those organisms which multiply in the host is uncertain and not often addressed: the infecting dose is not known in natural situations.

Experimental studies, where the dose is known, are discussed, as well as different natural situations in which the relative dose can be inferred. The advantages and drawbacks of the various methods are debated. The following sections focus on two infections: salmonellae and malaria.

For salmonellae, natural infections are compared using indirect markers of dose: incubation period, attack rate and type of vehicle. No evidence of a dose-severity relationship is found for typhoid, whereas there is some evidence for such a relationship for the food-poisoning salmonellae. Analysis of typhoid volunteer data suggests a dose-severity relationship; the critical role of illness definition in determining the findings is discussed.

Malaria therapy for neurosyphilis provides a unique source of information on large numbers of human subjects in whom a disease was induced artificially. An extensive review of the malaria literature provides no conclusive evidence on the relationship between dose and severity. The original records for patients from the Horton hospital in Epsom are analysed. Among 589 non-immune patients receiving vivax and 81 receiving ovale, who were not treated within the first 5 days, no consistent relationships are found between any direct measure of dose (mosquito number, sporozoite number, or trophozoite number) and any measure of severity including peak fever and parasitaemia levels. Dose is inversely proportional to prepatent period, and patients with longer prepatent periods are more likely to have tertian fever, undergo spontaneous recovery and not to get modifying treatment. The implications of these findings for the pathogenesis of malaria and for natural malaria are discussed.

The final section explores more general issues of the relationship between dose and pathogenesis.

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ACKNOWLEDGEMENTS

I would like to thank my supervisor, Professor David Bradley, for drawing my attention to the problem of the relationship between dose and severity and for his help and advice throughout the preparation of the thesis.

I am very grateful to the Wellcome Trust for awarding me the Training Fellowship in Epidemiology which made work for this thesis possible.

I would like to thank those researchers who generously gave me access to their data: Dr Stephen Palmer of the PHLS in Cardiff for the *Salmonella typhimurium* outbreak data; Dr Myron M Levine of the Center for Vaccine Development, Baltimore for data on volunteer studies with enteric infections; Dr WE Collins of CDC, Atlanta for the data on falciparum malaria. The researchers who maintained the Horton malaria records I thank, for the most part, posthumously and anonymously. The vision of Colonel James and others in seeing the role malaria therapy could play in furthering knowledge of malaria led to the detailed recording of treatments and outcomes which made my analysis of the malaria data possible. I would like to thank the librarians who allowed me easy access to the records, both at the London School of Hygiene and Tropical Medicine, and at the Royal College of Physicians, and the staff of the Horton Hospital, Epsom for allowing me to search through their notes.

Many people have contributed to this work through formal and informal questions and comments. I am particularly grateful to Dr Jo Lines for stimulating discussions on impregnated bednets and dose, Professor Paul Fine for comments on the Discussion, and Jamie Robinson for fielding statistical queries and for making sharing an office a rewarding experience. I would also like to thank Peter Aaby, Gustavo Bretas, Chris Dye, Alan Glynn, Ian Glynn, Andy Hall, Richard Hayes, Damien Jolley, Dave Leon, Sylvia O'Donnell, Jo Schellenberg, Brian Southgate, Carol Thacker, and Brian Williams.

Finally, I would like to thank my husband, John Twigg, for his enthusiasm for my work and his willingness to commute. I dedicate this thesis to him.

ABBREVIATIONS USED

ID50	The dose which infects 50% of those challenged
LD50	The dose which kills 50% of those challenged
r	Correlation coefficient
b	Regression coefficient
a	Intercept on the Y-axis in a regression equation
n	Number of subjects
SD	Standard deviation
SE	Standard error
CI	Confidence interval
Logit	Log odds. Logit x = log (x/1-x) where x is a proportion
β	Coefficent in a logistic regression equation
LRS	Likelihood ratio statistic
df	Degrees of freedom
AR	Attack rate. No. of cases/No. of susceptibles
CFR	Case fatality rate. No. of deaths/No. of cases
HR	Hospitalization rate. No. hospitalized/No. of cases
RR	Relative risk

INTRODUCTION

The occurrence of infection or disease following exposure of a host to a pathogen depends on three factors: (1) host characteristics, such as genetic factors and immune status; (2) pathogen characteristics (virulence); (3) factors affecting the interaction of the pathogen and the host (especially the dose of the pathogen). The same three factors may have a role in determining severity of disease once it occurs, although the third is often relatively ignored in this context.

Textbooks of infectious disease (eg Mandell et al 1990, Gorbach et al 1992) devote long sections to descriptions of microbial virulence factors and host defence mechanisms, but the relationship between host and microbe is restricted to a discussion of the distribution of diseases, transmission routes and establishing causes of cases. Infective dose is considered only as "the number of organisms necessary to cause an infection" (Mandell et al 1990). We expect severity of disease to depend on host resistance and the virulence of the strain of pathogen involved. For those pathogens which multiply in the host, once infection or disease is established, does the initial infecting dose continue to have an influence?

Among the helminth infections in which the parasite does not proliferate within the host, the expectations of a relationship between dose and severity are clearer and the evidence is easier to obtain. Where there is no proliferation, the worm burden in the host should depend on the initial dose. Correlations between inocula and worm load have been demonstrated in natural infections with *Ascaris lumbricoides* and *Trichuris trichiura* (Wong et al 1991). (A simple relationship between inoculum size and burden cannot always be assumed however: in experimental infections of pigs with *Ascaris suum*, larger inocula gave rise to the establishment of fewer adult worms (Andersen et al 1973).) Worm burden can be estimated either directly from the number of worms passed after treatment or indirectly from egg counts, though the egg production per female worm is higher when smaller numbers of worms are present (Croll et al 1982). Worm and egg numbers have been related to severity of disease. Thus hookworm egg counts have been related to the degree of anaemia (Stoll & Tseng 1925, Roche & Layrisse 1966), and correlations have been found between egg counts and severity of illness for schistosomiasis mansoni (Cook et al 1974) and schistosomiasis haematobium (Forsyth & MacDonald 1965). In trichuriasis, worm load correlates with the amount of faecal blood loss (Layrisse et al 1967) and larger egg counts are associated with more diarrhoea, dysentery and growth stunting (Jung & Beaver 1951, Cooper et al 1986).

Among the pathogens which do proliferate in the host it is both less clear that dose would be expected to influence severity, and harder to study the relationship, since most of the host

burden of pathogen will have arisen from subsequent multiplication.

More is known about the effects of dose in the stage of establishment of disease: attack rates and incubation periods have both been correlated with infecting dose (Sartwell 1966, Armenian & Lilienfeld 1983, Esrey et al 1985). The evidence for this comes principally from experimental infections in animals and humans. For example, human volunteer experiments have found increasing attack rates and/or decreasing incubation periods with increasing challenge doses of typhoid (Hornick et al 1970), food poisoning salmonellae (McCullough & Eisele 1951 a,c), cholera (Hornick et al 1971), enteropathogenic *E coli* (Levine et al 1978), Q fever (Tigertt et al 1961), and malaria (Coatney et al 1950b). Some studies have failed to find a relationship, but the number of volunteers with each strain of pathogen is often small (eg Mahonney et al 1946, Havens 1946). In animal experiments the response recorded is usually death and dose is recorded in terms of the LD50, the dose required to kill 50%. With increasing doses the mortality rate increases and the latent period to death decreases (Meynell & Meynell 1958). Further evidence comes from observational data. For example, Sartwell (1966) noted longer median incubation periods for serum hepatitis occurring after infections transmitted by contaminated vaccines than those transmitted by contaminated blood or blood products. Other examples are given in Section 1 in relation to indirect methods of assessing dose.

Whether, having influenced the probability of infection or disease and the time at which they occur, the initial infecting dose continues to have an influence on subsequent events for those organisms which multiply in the host is largely unknown and not often addressed. Such information as there is has not been brought together, and the subject is not easy to look up: "dose" or related terms are not generally indexed, and dose-response effects may be mentioned in the context of studies with other aims. Knowledge of dose-severity relationships is, however, important, and not just in furthering our understanding of the pathogenesis of infectious diseases.

The relationship is important in public health. If dose does determine severity, then an intervention which lowers infecting dose would usually be expected to be more effective against severe disease than against total disease. For example, a sanitary intervention which succeeded in lowering the dose of infecting organisms might decrease severe diarrhoeal disease while having little impact on mild disease (Esrey et al 1985) and this has obvious implications both for establishing and evaluating public health programmes. The relationship would also be

relevant in assessing the usefulness of a partially effective vaccine. A sporozoite vaccine for malaria which only succeeded in removing a proportion of the sporozoites might only reduce total disease slightly, but if there is a dose-severity relationship it might make severe disease much less likely.

If a dose-severity relationship is not considered, factors which may influence dose may not be recorded and biases introduced by differences in dose will be missed (Hall & Aaby 1990). For measles, Aaby (1988) has shown that secondary cases in a household have more severe disease than index cases and this is likely to be due to differences in infecting dose. (The evidence for this is discussed in Section 1.) A study of measles in women in Los Angeles concluded that pregnancy increased the risk of hospitalization, pneumonia and death (Eberhart-Phillips et al 1992). However, this study did not consider whether these women were primary or secondary cases in their households, and pregnant women, who may be more likely to have families with young children, may be consequently more likely to be secondary cases. Since the question was not considered, the influence (if any) of this potential confounder on the results cannot be assessed. Bias arising from a failure to consider dose as a possible risk factor for severe disease is likely to be a particular problem in community trials where there are few units of randomization, making chance differences between groups in factors influencing dose, such as household size, more likely (Hall & Aaby 1990).

In this thesis the dose-severity relationship will be considered both in general terms and in relationship to particular diseases. Since a major reason that infecting dose is often neglected is that the dose is rarely known (Greenwood 1987), the first section will consider different situations in which the dose is known or can be inferred. I consider a range of experimental and observational methods of assessing dose-severity relationships, giving brief examples of their use and exploring their advantages and disadvantages. I give more weight to the observational methods because they are less obvious methodologically, they have potentially greater scope, and they create greater problems in interpretation. In the following two sections I apply some of these methods to the study of two very different infections, salmonellae and malaria. These sections include both literature reviews and analysis of raw data. Finally, I discuss the theoretical relationships between dose and the pathogenesis of disease.

SECTION 1

METHODS OF INVESTIGATING DOSE-SEVERITY RELATIONSHIPS

Two main methods exist for determining the relationship between infecting dose and severity: experimental and observational. In experimental situations, known doses of pure strains of organisms are given to animals or human volunteers under controlled conditions. Using observational methods, indirect measures of dose or relative dose are explored in natural situations. Experimental methods can provide "clean" data but are likely to be available for only a few infections and the results are not easy to generalize. Observational methods are necessarily indirect and are subject to a range of biases, but the results can be more directly applied.

ANIMAL EXPERIMENTS

Animal experiments have several advantages as well as the ability to control the inoculum size. Other variables such as environmental conditions, diet and genetic factors can all be tightly controlled, invasive measures of disease are possible, and infections can be observed untreated. In relation to pathogenesis, it can be particularly useful to observe the microbial load and the effects of disease in different organs. On the other hand there are numerous disadvantages, including the obvious difficulties of extrapolating findings in animals to people. Since the purpose of animal experiments is usually to further knowledge about human disease, the infections studied in animals are often not those which occur naturally in the species. Very large inocula are often used. These may be necessary to induce disease in the animal species, or at least to produce high response rates so that few of the inoculated animals are "wasted". For similar reasons inoculation often occurs by unnatural routes such as intraperitoneal or intracerebral injection.

In the small animals commonly used for experiments the only measurable outcome is often death. In these systems attack rate can be determined but not severity. The time to death may be recorded, but this reflects the incubation period rather than the severity of disease - which is, after all, the same in each case. It may also be possible to distinguish, for example from blood or stool samples, whether infection is established, thus giving two measures of outcome. But infection is not the same as disease, and it is levels of severity of symptomatic disease which are at issue here. This point is explored further in the Discussion.

In larger animals other measures are possible, but experiments are often very limited in size. When the dose is varied, often only a few animals receive each dose. These experiments are

small for reasons of expense, logistics, and ethics. (Ethical considerations obviously influence all experimental infections: in this thesis I am considering experiments already carried out by other people.)

Although a major concern in animal experiments is often their artificiality, occasional experimenters move so far in the other direction, creating conditions whereby animals naturally transmit disease from one to another, that information on dose is lost. Thus in Greenwood et al's experimental epidemics in mice (1936), in which they created communities with shifting populations and watched epidemic spread, no more information on dose is provided than by natural epidemics. However, in Lurie's experiments with tuberculosis in rabbits (1964) three different relative doses could be deduced, despite fairly natural transmission from infected animals: from the proportion of infecting rabbits with tubercle bacilli in their urine, the type of bedding used, and whether ultraviolet irradiation was used.

This work by Lurie (1964), designed to study mechanisms of resistance, highlights the potential problems in drawing conclusions on the effects of dose from experimental situations. Using controlled measures for determining inhaled dose from an aerosol he found, with bovine tuberculosis in rabbits, that the larger the infecting dose, the more fulminating the disease and the shorter the survival. These artificially infected animals had numerous primary foci in their lungs; indeed, in those infected by 2000-3000 bacilli the primary foci were so numerous that death occurred from simple consolidation of the lung. Even with small doses multiple foci occurred, on average one focus for every three highly virulent bacilli (representing the proportion of the inoculum reaching the alveoli). This is a marked contrast to the single focus usually found in rabbits (and people) who contract disease from exposure to tuberculous companions. Here, despite using the correct route of infection, and doses as low as 23 bacilli per rabbit, the disease produced was different in nature to that occurring following rabbit to rabbit transmission.

Many animal experiments use unnatural routes of infection and this can affect the results. Standfast and Dolby (1961) demonstrated different patterns of growth of *Bordetella pertussis* in mice when the bacteria were inoculated intranasally or intracerebrally. Intracerebral inoculation of monkeys with polio virus has demonstrated that larger doses produce more severe paralysis (Levinson et al 1945) but the relevance of this for an orally transmitted disease is difficult to ascertain. In studying host-parasite relationships in terms of bacterial dose, Meynell and Stocker (1957) argued that the stage of the challenge dose managing to penetrate the host was

"less interesting" than the next stage, within the host tissues, and so used intraperitoneal injections for many of their experiments. However the dynamics of the first stage could affect not only the dose but also the host response and the pathogenesis in the next stage, so to draw conclusions about natural infections, natural routes of infection are preferable.

In some cases the disease in the animal is the focus of interest in itself. Here expense is the major limitation as, in commercial terms, it is generally only the diseases of expensive animals which are worth investigating. For example, equine influenza was studied in ponies challenged by aerosol inhalation (Mumford et al 1990). Only 3 to 6 ponies were used in 4 dose groups and only 12 became ill. With increasing dose the incubation period decreased and the duration of viral excretion increased. The mean temperature of those with fever was higher in the highest dose group than the others.

HUMAN EXPERIMENTAL STUDIES

Human experiments share the advantage of all experimental studies that the inoculum size can usually be fixed with some accuracy. It is possible to control some other variables, for example the age and general health status of the subjects and the vehicle of infection, and to measure others, such as gastric acidity, so that the analysis can be adjusted accordingly. They provide direct information on human diseases in their natural hosts. Subjects are usually infected by natural routes although not necessarily in naturally occurring doses. The major limitations derive from ethical constraints.

The scope of human experiments is very limited in terms of both the different diseases studied and the number of subjects employed. The main reasons why human studies employing varying doses have been carried out are to establish whether certain organisms are pathogenic (eg McCullough & Eisele 1951a,c,d), to establish a challenge dose for future vaccine studies (eg Hornick et al 1970, Cash et al 1974) or to investigate possible routes of transmission (eg Havens 1946). The outcome of interest in each case is whether the subject becomes ill or not; severity of illness, if it is considered at all, is secondary, and patients are often treated early.

Because severity of illness is not the outcome of interest for the authors, the information presented is often incomplete. For the studies carried out on human volunteers at the Center for Vaccine Development at the University of Maryland, Baltimore, I have inspected the original data for those infections where more than one dose of a single strain of organism was given to unvaccinated volunteers. Some, but not all, of this data has been published. Information was available for infections with *Campylobacter jejuni*, *Shigella flexneri*, *E coli*, and cholera. The methods are described in the published accounts mentioned below. Briefly, volunteers were kept in an isolated ward, randomised to dose, vaccination and treatment groups and challenged with the stated inoculum in a standard vehicle. All stools were collected, inspected and measured and other symptoms and temperatures were recorded. In general I have used summaries of the data rather than day to day accounts of the symptoms in order to extract the data given below. I have only used data from volunteers who had neither been vaccinated nor received previous challenges; they were often the controls for other studies. Earlier work by the same group, much of which has been published, was carried out in prisoner volunteers. I have included this in the descriptions as appropriate.

Campylobacter jejuni

The information for *Campylobacter jejuni* has already been published (Black et al 1988). With strain A3249 the proportion of volunteers with positive stool cultures increased with increasing dose, but the proportion with symptoms showed no consistent relationship with doses ranging from 800 to 10^8 organisms. Overall only 13/72 were symptomatic. With strain 81-176 in doses from 10^6 to $2x10^9$, all 39 volunteers had positive stool cultures. Eighteen volunteers were ill, and again the attack rate did not appear to be dose-dependent. For neither strain was there a correlation between the challenge dose and the mean number of liquid stools passed nor the mean diarrhoea volume in ill volunteers.

Shigella flexneri 2a

Information is available on challenges using strain 2457T in 339 volunteers challenged over a period of more than 20 years. Most of the information is published (Dupont et al 1969, 1972, Levine et al 1977) and information on these earlier challenges is only available to me in the published accounts. The data is summarised in Table 1.2.1. In the two earlier reports, the lowest dose group had lower attack rates, but the lack of effect of dose on attack rate is more striking and there is little evidence of a correlation between dose and severity.

Escherichia coli

For all the available data with *E coli*, published and unpublished, the number of volunteers becoming ill at each dose with each strain is very small. The published data concerns mainly enteropathogenic (EPEC) strains, and the unpublished data mainly enterotoxigenic (ETEC) strains.

Information relating to different challenge doses is available for 4 strains of EPEC all derived from diarrhoea in infants (Levine et al 1978, 1985). With doses ranging from 10^6 to 10^{10} almost all of the volunteers developed positive stool cultures and the attack rates for illness increased with dose. Measures of severity are only available for two of the strains. For strain E128010 (Levine et al 1985), diarrhoea stool volumes were similar for the 3 volunteers becoming ill after challenge with 10^8 organisms and the 3 ill after challenge with 10^{10}. For strain E851/71 (Levine et al 1978 and unpublished data), 1/5 volunteers became ill after a dose of 10^6 organisms after an incubation period of 63 hours and had 6 diarrhoea stools totalling

437ml; 1/5 became ill after 10^8 organisms after 10 hours and had 2 diarrhoea stools totalling 459ml; all 5 volunteers receiving 10^{10} organisms became ill with a mean incubation period of 12 hours (range 9-13), had a mean of 5 diarrhoea stools (range 3-9), totalling a mean of 1015ml (range 681-1403). Three in the high dose group also had vomiting.

For ETEC the available data on unvaccinated volunteers, almost all of it unpublished, is summarised in Table 1.2.2. Data are presented for all the strains where there were at least 2 ill volunteers in at least 2 groups and some measures of severity were available. Strain H-10407 is typed as O78:K80:H11 and produces heat labile and heat stable toxins (abbreviated as LT+/ST+). It was originally isolated from a patient with copious diarrhoea in Bangladesh. It has also been studied by Satterwhite et al (1978). Strain B7A (O148:H28) was originally isolated from a soldier in Viet Nam, is also LT+/ST+ and is described by Levine et al (1979). Strain TD 225-C4 (O75:H9) came from a patient in Mexico City and is LT+/ST-. Strain 350-C1 (O159:H4) came from a patient in Kenya and is LT+/ST+. All challenges were given in a standard concentration of sodium bicarbonate. Antibiotics, usually neomycin, were usually given at 4 or 5 days, depending on the exact protocol used. Since neomycin can cause diarrhoea all symptoms recorded are those occurring before the antibiotic.

Stool cultures were positive on almost all of the volunteers and attack rates generally increased with dose. There were no clear relationships between dose and severity of disease, measured in terms of number of diarrhoea stools passed or total volume of diarrhoea, but the numbers in each group were small (Table 1.2.2).

Cholera

Much of the cholera data has already been published. Results from apparently the same prisoner studies are given in three papers: Music et al 1971, Hornick et al 1971 and Cash et al 1974. An amalgamation of their figures is given in Table 1.2.3. All doses were given with bicarbonate. The attack rate increased with dose and for Inaba 569B the proportion of subjects with diarrhoea who required intravenous therapy increased with increasing dose (χ^2 trend 4.9, $p = 0.03$).

Much of the more recent cholera data has also been published. The data available to me (in the form of experiment summaries) is presented in Table 1.2.4. The results using El Tor Inaba P27459 and N16961 appear (in slightly different form) in Levine et al 1981. Both strains were

isolated from patients with cholera in Bangladesh. The classical strains were the same as those used in the earlier prisoner studies; both were originally isolated from cholera patients in India (Cash et al 1974).

The challenge doses were given with 2g of sodium bicarbonate but it has been demonstrated (Music et al 1971) that 30 minutes after 2g bicarbonate the stomach pH in about half the investigated subjects becomes strongly acid again and in the other half remains near neutral, with no overlap between the groups. Those with prolonged buffering have much higher attack rates when challenged with cholera given with bicarbonate. Nalin et al (1978) showed that basal stomach acid production 24 hours before ingestion of cholera with bicarbonate correlates with disease severity, those with low acid having higher total volumes of diarrhoea. It is also known that blood group O predisposes to more severe disease (Levine et al 1981). The volunteers were randomised to the different groups, but as the numbers are small it is quite possible that the proportions with blood group O and low acid secretion were not evenly distributed between the groups. I have no information on the acid secretion in these volunteers, and the information available to me on blood groups is very incomplete so I have not been able to examine the effects of these factors in the results.

The data for the classical strains (Table 1.2.4) provide no evidence that higher doses lead to more severe disease - in contrast to the earlier studies with the same strains (Table 1.2.3). For Classical Inaba 569B, over the small dose range studied, there was little difference in severity between the groups. For Classical Ogawa 395 three of the four volunteers ill after receiving the lower dose, 10^5 organisms, had particularly severe disease, with very large stool volumes. The differences between the two dose groups were significant at the 5% level for the stool volume, the number of diarrhoea stools and the proportion of ill subjects with rice water stools (comparison of means using Kruskal Wallis non-parametric test and proportions with Fisher's exact test).

For El Tor Inaba P27459 those receiving the lower dose again had larger stool volumes (p = 0.03 Kruskal Wallis non-parametric test comparing group means). Other measures of severity were similar in the two groups. For El Tor Inaba N16961, as the dose increased the incubation period decreased, and the diarrhoea stool volume and number increased. These continuous variables were inspected on scatter plots and linear regressions were calculated between log dose and log incubation period, log stool volume and log number of stools. The following correlations with log dose were obtained: log incubation period correlation coefficient (r) =

-0.40 (95%CI -0.70 to 0.03); log stool volume r = 0.33 (-0.11 to 0.66); and log number of stools r = 0.22 (-0.22 to 0.59). With increasing dose the proportion of ill subjects with rice water stools increased (χ^2 trend 5.7, p = 0.02).

Overall the lack of correlation between dose and severity is striking, even when the dose varies by three logs. Gastric acidity is known to have a major effect on outcome, and it would be very interesting to adjust the results by basal acid secretion, to see if this is masking any effect of dose. The dose arriving in the small intestine could be crucial.

Table 1.2.1. Comparison of outcome in non-immunised volunteers challenged with different doses of *Shigella flexneri 2a*.The symptoms are given as a proportion of ill volunteers.

Source	Dose	N	Ill (%)	Stool +ve (%)	Fever	Diarrhoea	Dysentery
Dupont et al 1969	10^4	4	1 (25%)				
	10^5	4	3 (75%)				
	10^6	8	7 (88%)				
	10^7	19	13 (68%)				
	10^8	8	7 (88%)				
total	10^4-10^8	43	31 (72%)	29 (67%)	13/31 (42%)	27/31 (87%)	16/31 (52%)
Dupont et al 1972	180	36	9 (22%)	6 (17%)			
	5000	49	28 (57%)	33 (67%)			
	10^4	88	52 (59%)	66/87 (76%)			
	10^5	24	14 (58%)	15 (63%)			
Levine et al 1977	100	36	14 (39%)	12 (33%)	7/14 (50%)	14/14 (100%)	8/14 (57%)
	10^4	15	6 (40%)	9 (60%)	3/6 (50%)	5/6 (83%)	5/6 (83%)
Unpublished data, 1986 to 1991	100	8	4 (50%)	5 (63%)	3/4 (75%)	3/4 (75%)	4/4 (100%)
	10^3	40	21 (53%)	20/28 (71%)	17/21 (81%)	18/21 (86%)	19/21 (90%)

The definitions of ill, fever and diarrhoea all vary:

- Dupont et al 1969: Ill = fever (≥ 100°F), severe abdominal cramping, diarrhoea (≥ 2 loose stools/24 hours), or bloody mucoid stools.
- Dupont et al 1972: Ill = fever (≥ 100°F) + diarrhoea (≥ 4 watery stools/24 hours)
- Levine et al 1977: Ill = diarrhoea (≥ 3 loose stools/24 hours) or dysentery ± fever (≥101°F)
- Unpublished: Ill = diarrhoea (≥ 1 liquid stool ≥ 300ml or ≥ 2 liquid stools totalling ≥ 200ml within 48 hours) or dysentery ± fever (≥ 100°F)

Dysentery = blood and mucus in stool

Table 1.2.2. Summary of unpublished data from the Center for Vaccine Development, Baltimore, for different strains of ETEC. (Some of the data for strain B7A is published in Levine et al 1979)

Strain	Dose	N	Stool +ve	Ill (%)	Incubation period (hours) Median (range)	No. of diarrhoea stools. Median (range)	Volume of diarrhoea stools (ml) Median (range)	Severe
H-10407	10^6	5	5	1 (20)	70	7	552	0/1
1978-92	10^8	5	5	2 (40)	50 (48-52)	6 (2-10)	717 (347-1087)	1/2
	10^9	34	34	25 (74)	43 (21-92)	8 (1-39)	1290 (40-6100)	10/25
H-10407 clone	5×10^6	6	0	0 (0)				
	10^7	11	11	3 (27)	31 (23-49)	15 (4-26)	1516 (410-1536)	1/3
1979-80	5×10^8	21	21	19 (90)	40 (23-78)	11 (1-29)	1762 (30-9860)	12/19
B7A	10^6	6		3 (50)	25 (23-52)	8 (6-10)	795 (640-1300)	0/3
1977-80	10^8	27	3/3	17 (63)	44 (14-124)	6 (1-25)	600 (300-2150)	4/17
	10^{10}	13	12	8 (62)	15 (9-48)	5 (2-24)	580 (218-3311)	3/8
TD 225-C4	10^6	5		1 (20)	1	3	40+	0/1
1977-80	10^8	4		3 (75)	7 (2-8)	4 (3-6)	326 (281-595)	0/3
	10^9	3		2 (67)		5 (3-6)	656 (315-997+)	1/2
	10^{10}	5	5	2 (40)	10 (8-12)	4 (2-5)	576 (393-759)	0/2
350-C1	10^6	3	3	0 (0)				
1987	10^8	4	3	0 (0)				
	10^9	4	4	2 (50)	19 (12-26)	1 (1-1)	272 (189-354)	0/2
	10^{10}	5	5	3 (67)	18 (15-21)	2 (2-3)	206 (10+-403)	0/3

A volunteer was counted as ill if they had diarrhoea (ie stools which take the shape of the container) fulfilling at least one of the following criteria:

1 diarrhoea stool ≥ 300ml or 2 diarrhoea stools totalling ≥ 200ml or ≥ 3 diarrhoea stools or any amount of diarrhoea + vomiting or fever (≥ 100°F)

Severe illness was defined as diarrhoea stool ≥ 2l or ≥ 1l + vomiting or fever

Table 1.2.3. Results of volunteer challenges with two strains of classical cholera. All challenges were given with bicarbonate. From Music et al 1971, Hornick et al 1971 and Cash et al 1974.

Strain	Dose	No. of volunteers with each outcome			
		Nil	Carrier	Diarrhoea	Severe diarrhoea
Inaba 569B	10	2	0	0	0
	10^3	1	3	0	0
	10^4	2	2	9	0
	10^5	1	1	5	1
	10^6	4	6	28	14
	10^8	0	0	1	1
Ogawa 395	10^3	1	0	1	0
	10^6	2	0	11	9

The outcomes are defined as follows: Nil - stool cultures negative; Carrier - stool culture positive, no diarrhoea; Diarrhoea - stool positive with at least one diarrhoea stool but not requiring intravenous fluids; Severe diarrhoea - stool positive with diarrhoea severe enough to require intravenous therapy.

Table 1.2.4. Summary of results of cholera challenges in non-immunized volunteers at the Center for Vaccine Development, Baltimore. All challenges were given with bicarbonate.

Strain	Dose	N	Stool +ve	Ill (%)	Incubation period (hours) Median (range)	Stool volume (ml) Median (range)	No. of diarrhoe a stools Median (range)	Rice water stools
El Tor Inaba P27459 (1978)	10^5	5	5	3 (60)	25 (17-41)	3049 (1642-9856)	11 (8-18)	1/3
	10^8	11	11	9 (82)	24 (18-50)	1313 (324-2755)	10 (3-28)	3/9
El Tor Inaba N16961 (1978-82)	10^3	6	6	4 (67)	32 (29-40)	687 (422-1876)	6 (2-9)	0/4
	10^4	5	4	4 (80)	39 (29-40)	1074 (590-1526)	6 (4-10)	0/4
	10^5	5	4	3 (60)	19 (17-19)	2489 (2224-4656)	15 (9-21)	1/3
	10^6	12	11	11 (92)	24 (14-55)	2751 (280-13100)	12 (2-39)	6/11
Classical Inaba 569B (1990-91)	1.5×10^6	13	10	5 (38)	33 (19-60)	874 (394-1897)	6 (2-12)	0/5
	4×10^6	15	15	10 (67)	51 (29-90)	712 (227-1741)	6 (2-15)	0/10
	10×10^6	11	9	8 (73)	32 (16-72)	794 (214-2810)	7 (2-16)	0/8
Classical Ogawa 395 (1977-80)	10^5	6	6	4 (67)	39 (19-48)	20940 (1410-44020)	46 (9-87)	4/4
	10^6	28	27	26 (93)	30 (7-104)	3050 (200-18270)	18 (1-41)	9/26

Volunteers were classified as ill if they had positive stool cultures and diarrhoea (defined as a liquid stool - ie one which takes the shape of the container) of ≥ 300ml or two liquid stools totalling ≥ 200ml within 48 hours.

OBSERVATIONAL STUDIES

Observational studies rely on indirect measures of dose. Because they refer to natural infections any results are more directly relevant to public health than those from experimental studies. Information is potentially available on a wide range of diseases, and very severe disease or death, if it occurs, can be studied as an outcome.

The main disadvantages are that only proxy and imprecise measures of dose are available, measures of severity are often crude, and the data are often of poor quality. There are inherent biases in many of the methods, related to the proxy measures used. Methods of assessing dose or relative dose are the major problem. Some methods can be used for a range of infections but many are dependent on the route of infection.

General methods

Attack rate and incubation period

The attack rate and incubation period are associated with dose for many infections. As discussed in the introduction, the evidence comes mainly from animal and human volunteer experiments. An outbreak with a high attack rate and a short average incubation period is likely to have resulted from a high dose of infecting organisms. Of course alternative explanations are possible: the outbreak could be due to a particularly virulent strain of organism, or the population could be particularly susceptible. Nevertheless, comparing attack rates or incubation periods between outbreaks caused by the same organism provides a proxy method of assessing dose. I have used these methods in comparisons of typhoid and of salmonella outbreaks (Section 2, and Glynn & Bradley 1992).

Within a single outbreak the problem of confounding by strain can be ignored. Attack rates by area may occasionally be available in large epidemics (Bernard 1965, Shaw 1922), allowing attack rates to be compared to a measure of group severity such as the case fatality rate. More detailed information can be obtained by comparing individual incubation periods with individual outcomes and this method is more widely applicable. Incubation periods can be estimated from point source epidemics so the method is especially suitable for food or water-borne diseases. I have carried out a detailed analysis of a *Salmonella typhimurium* outbreak using this technique (Section 2, and Glynn & Palmer 1992). Other applications are possible.

For example, an outbreak of illness, later identified as histoplasmosis, affected all 21 people who had been in a cave during a treasure hunt in Arkansas in 1947 (Washburn et al 1948, Grayston & Furcolow 1956). Incubation periods were recorded and did not correlate with disease severity. To give another example, the time between exposure and the onset of prodromal symptoms was recorded for 37 children with a known single exposure to polio in an epidemic in Alabama in 1941 (Casey 1942). There was no association between the incubation period and the outcome, categorized as non-paralytic, paralytic or death.

Incubation periods may also be known for individuals outside single outbreaks or in more prolonged epidemics which may not necessarily be due to a single strain. It is still possible to compare the incubation period with the outcome, but possible confounding by strain differences should be considered.

The inherent bias in using incubation period as a measure of dose is confounding by individual susceptibility. Host immunity and innate susceptibility would be expected to influence both the incubation period and the severity of disease, leading to an association which could be wrongly interpreted as evidence of a dose-severity effect. That an association between incubation period and severity does not necessarily imply a dose effect is illustrated by data from the Center for Vaccine Development, Baltimore. Among volunteers, about 40% of whom had been vaccinated, challenged with an identical dose of *Shigella sonnei*, incubation period was inversely related to the peak temperature (Mackowiak et al 1992). In the cholera data from unvaccinated volunteers discussed above (Table 1.2.4), incubation period was more closely correlated with outcome than was dose. For example, for El Tor Inaba N16961 where there was a large dose range, log stool volume was more closely related to log incubation period ($r = -0.65$, 95% CI -0.84 to -0.32) than to log dose ($r = 0.33$, 95% CI -0.11 to 0.66). Here the incubation period may reflect the dose reaching the small intestine as well as individual susceptibility to the effects of the toxin.

In some situations it is possible to adjust for factors known to influence susceptibility, such as age, prior exposure or vaccination, or the use of antacids and antibiotics. This is discussed further in the sections on salmonella and malaria. Residual confounding by known and unknown or unmeasurable factors is likely.

Observing the effect of an intervention

Another method of estimating the effects of different relative doses might be to assess the

effects of an intervention aimed at lowering infecting dose. As emphasised in the introduction, if there is a dose-severity relationship, an intervention which lowers the dose would be expected to be more effective against severe disease than against mild disease (Esrey et al 1985). To use this to investigate the effects of dose we would have to know what would happen to the protective efficacies against different levels of disease if there were no dose-severity relationship, which supposes a knowledge of the shape of the dose-response curve for the different levels of disease.

The shape of the curve can be predicted (Meynell & Stocker 1957) for a single pathogen given by a uniform method of inoculation to hosts of uniform resistance, assuming that the organisms in the inoculum act independently of each other. Where p is the probability that each organism gives rise to disease and d is the infecting dose, the probability of a given host not getting the disease is $(1 - p)^d$.

The probability of responses to different doses under these assumptions is shown in Figure 1.3.1. If it is assumed that the probability that each organism giving rise to severe disease is smaller than its probability of giving rise to disease, then the probability of severe disease can be represented by the curve on the right. An intervention which lowers the dose received will lower the probability of getting both disease and severe disease but the protective efficacies against these different outcomes will not necessarily be the same - it will depend on the relation between the reduction in dose and the different parts of the two curves. In fact the protective efficacy will always be greater against the less likely event although the difference is sometimes negligible.

This very simplified model suggests that, following an intervention which lowers infecting dose, the protective efficacies against different levels of disease cannot be assumed to be the same even in the absence of a dose-severity relationship. Aspects of this simple model of infectious disease are discussed in the final section. In practice, the shape of the dose-response curve even for the level of illness which defines disease is not well established for most pathogens. It will depend on the pathogen, the method of inoculation and the hosts (Esrey et al 1985). The finding that an intervention has a greater protective efficacy against severe disease than total disease in a study would therefore not necessarily imply that a dose-severity effect existed. Other alternative explanations are also possible, and the subject is explored further in relation to impregnated bednets and malaria (Section 3).

Food- drink- or water-borne transmission

Amount of food

Where the infecting organism is uniformly distributed in a food vehicle, and the food is eaten over a short space of time, the amount of food consumed should provide a good proxy marker of relative dose. This relationship can be used practically in investigating outbreaks: an increase in attack rate with increasing doses of a particular foodstuff can aid identification of the vehicle. For example, in an outbreak of disease due to *Campylobacter jejuni* following a school visit to a farm in Minnesota (Korlath et al 1985), the attack rate increased with increased raw milk consumption. However, among the 25 who were ill in this outbreak, there were no apparent associations between the amount of milk drunk and either the incubation period or the duration of illness.

In an outbreak of Hepatitis A traced to tuna sandwiches prepared by an infected person, all 7 people who ate the sandwiches became ill, and the incubation period was inversely related to the number of sandwiches eaten (Istre & Hopkins 1985). In this small outbreak no associations were found between dose and severity of disease in terms of duration of jaundice, number of symptoms or time off work.

Examples relating to salmonella and typhoid are discussed in Section 2. Unfortunately the amount of food is rarely recorded, and even when it is, the range of dose is likely to be small and the distribution of the pathogen in the food is unlikely to be uniform. People who eat larger amounts of the affected food may tend to eat larger (or smaller) amounts of another food which may be an effective buffer, or may differ in other ways from people who eat less, so the relationships between the amount of the vehicle consumed and the outcome may not be straightforward. Another potential problem in this type of study is recall bias. Since, intuitively, people may expect that larger doses will make them more ill, if the vehicle is known, differential misclassification of exposure may result.

The time when food is eaten

If a foodstuff is contaminated with bacteria and left at a temperature at which the bacteria can multiply, then food eaten later is likely to be more heavily contaminated. Again this is not often recorded: some examples of outbreaks where food was eaten at different times are given in Section 2.

Type of vehicle

It is likely that the concentration of pathogens in water is usually lower than that in food (Naylor 1983). For pathogens that can be transmitted by either food or water it is possible to compare outbreaks caused by the different vehicles. In Section 2 this has been done for typhoid, and the possible drawbacks of this approach are discussed.

Blood transmission

Whole blood or concentrated blood products

Blood products such as factor VIII concentrate are made by pooling blood from many donors and then dividing up the product between recipients. Blood products were not routinely heat-treated until 1985. In contrast, single units of whole blood come from single donors. It is likely therefore that infected units of blood contain a higher concentration of HIV or Hepatitis B than do vials of concentrate.

For HIV, severity can be measured by progression to AIDS or by changes in markers associated with this progression over time (Moss 1988). Studies following cohorts with known dates of seroconversion have found variable rates of progression to AIDS. A Swedish study (Giesecke et al 1988) found a much higher rate of progression among blood transfusion recipients than among haemophiliacs. This might suggest that dose influenced progression, but the transfusion recipients were older, and although age did not predict progression within each group in this study, in other studies cohorts have shown faster progression among older patients (Darby et al 1989, Goedert et al 1989). The two groups - haemophiliacs and blood transfusion recipients - will always be difficult to compare since the illness for which the patients required the blood transfusion, or associated illnesses, may influence progression.

Number of vials of Factor VIII

The Edinburgh haemophilia cohort provides uniquely direct evidence relating to inoculum size (Cuthbert et al 1990). Thirty-two patients with haemophilia A received doses of a single batch of factor VIII contaminated with HIV-1. Eighteen seroconverted and the number of vials they received was recorded. The number of vials did not influence the time to seroconversion. Doses ranged from 9 to 109 vials: 7/9 receiving more than 30 vials reached CDC stage IV by 5 years, compared to 3/9 receiving up to 30 vials (relative risk 2.3, 95% CI 0.87 to 6.27).

It might be possible to determine other groups of haemophiliacs all infected from the same batch in whom the relative dose could also be compared. Several large cohorts of haemophiliacs for whom the time of exposure is known are reported in the literature (Giesecke et al 1988, Darby et al 1989, Goedert et al 1989, Ragni et al 1990, Eyster et al 1989, Lee et al 1989, Wolfs et al 1989, McGrath et al 1986). For some of these cohorts reports refer to types of blood product used, and Goedert et al (1989) in their multicentre cohort, extracted data on cumulative dose of factor concentrate, implying that these details are recorded. Both in the United States and in the United Kingdom it is possible to trace a particular factor concentrate batch to its recipients (Cuthbert et al 1990, Jason et al 1986).

For each individual in a cohort the batches and amounts of factor VIII received over the period before seroconversion could be identified. By comparing these results with the batches received by seronegative haemophiliacs from the same centre over the same period, it should be possible to determine which of the batches were infected (using methods analogous to those used to identify the vehicle in food poisoning outbreaks). Whether the number of vials of the infected batch influenced the outcome in groups all infected by a single batch could then be determined. This procedure would only be possible if, as in Edinburgh in 1984, the prevalence of HIV infection was low enough that each haemophiliac is likely to have been infected by only one batch.

Airborne transmission

Length or extent of exposure to an agent in the environment

The duration of exposure to a single airborne source of infective agent should correlate with infective dose. In the Arkansas outbreak of histoplasmosis discussed above (Washburn et al 1948), duration of exposure in the cave was positively associated with the severity of the outcome; however neither the definition of duration nor of severity were clearly stated and the incubation period was not related to the duration of exposure. In a review of histoplasmosis, which also lacks definitions, Grayston and Furcolow (1956) found that five severe epidemics followed exposure in enclosed situations, whereas for five less severe epidemics the situations were "open" for two, "partially open" for one, and "unknown" for two.

Exposure to more than one infective case

Studies in military populations have shown that the acquisition rates for Group A streptococci increased with the number of carriers in a barrack group (Wannamaker 1954), the higher infection rates suggesting higher infecting doses.

Neither Top (1938) nor Stillerman and Thalhimer (1944) found higher attack rates for measles among susceptible contacts exposed to more than one primary case in the family. In the later study there were only 9 such contacts, and in both studies the overall attack rates were around 80% and higher in the under fives so there was little scope for an increase. Both authors also considered duration of exposure and found little difference in attack rate between contacts exposed throughout the illness and contacts of those removed to hospital. However most transmission would be expected to have occurred before this time (Benenson 1990).

In a Kenyan study of measles (Aaby & Leeuwenburg 1990), exposure to more than one index case affected the outcome: secondary cases exposed to 2-4 index cases had a case fatality rate (CFR) of 5/37 (14%) compared to a CFR of 18/303 (6%) for those exposed to only one index case (RR 2.47, 95% CI 0.93-6.56, after adjusting for age).

Proximity to infective case

Studies of Group A streptococcal carriage in volunteers found that acquisition rates decreased with increasing bed distance from the nearest carrier (Wannamaker 1954). In an outbreak of scarlet fever in the Royal Naval School, Greenwich, Dudley (1923) found clustering of cases at one end of a large dormitory, and no cases among day boys. Other boys would have had contact with the infected cases at other times of day so this difference in attack rates suggests an effect of dose. The importance of proximity in effective transmission led to the development of rules for bed spacing to try to limit epidemic spread (Stallybrass 1931).

In Senegal (Garenne & Aaby 1990) secondary cases of measles living in closer proximity to the index cases had higher CFRs than those in separate huts and households but still within the same compound.

Index or secondary case in a household

Peter Aaby (1988, 1989) has argued that the secondary cases of measles in a household are exposed to a higher infecting dose than index cases infected outside and that this explains some of the variation in severity of this disease. Since 1978 a child health and nutrition project

has been running in Guinea-Bissau, where measles case fatality rates can reach 25%. The failure to find the expected association between pre-morbid nutritional status and outcome from measles infection led to a search for other explanations. It was found that cases occurring in households with more than one case had a higher fatality rate than isolated cases (Aaby 1988,1989). Other studies have been reanalysed to compare rates between multiple and single cases, and between index and secondary cases (Aaby 1989, Koster 1988, Bhuiya et al 1987, Garenne & Aaby 1990, Aaby & Leeuwenburg 1990, Pison & Bonneuil 1988, Lamb 1988, Hull 1988) and generally confirm the results, giving case fatality rate ratios between secondary and index cases of around 2 or more.

The results are very unlikely to be due to chance; many studies individually had statistically significant results at least for the younger age groups (Aaby 1989, Koster 1988, Bhuiya et al 1987, Garenne & Aaby 1990, Aaby & Leeuwenberg 1990). Ascertainment of measles cases was generally thorough - measles was well recognized in the communities (Garenne & Aaby 1990) and where antibody levels have been checked lay diagnosis has proved reliable (Aaby 1989, Koster 1988).

Younger children are more likely to be infected at home in many communities, and therefore become secondary cases; and younger age at infection has been widely shown to be associated with higher mortality (for example Hull 1988, Aaby 1989, Pison & Bonneuil 1988, Narain et al 1989, Gordon et al 1965). However the association persists within each age band, and is particularly strong in those under three years (Aaby 1988). In fact controlling for index/secondary cases may eliminate some of the association with age (Bhuiya et al 1987).

Large households will tend to have more secondary cases and are particularly subject to the problems associated with overcrowding such as poor nutrition and hygiene. However the increased case fatality rate among secondary cases is found when the analysis is restricted to families with one index and one secondary case (Aaby & Leeuwenberg 1990). It may be argued that families may be less able to look after the second sick child, but in Guinea-Bissau there was no lower mortality among secondary cases whose mothers only had to care for them, than among those whose mothers had to care for an index case as well (Aaby et al 1988).

The proportion of secondary cases in studies in different communities in different countries correlates closely with overall case fatality rates for those communities (Aaby 1989). The different proportions of secondary cases are presumably due to different family structures, in

particular the high rates of polygamy and extended families in West Africa, and to the local pattern of measles, whether it is endemic or epidemic. Secular changes in family size and living conditions in the developed world could contribute to the observed decrease in case fatality rates by changes in the proportion of secondary cases.

In urban areas where measles is endemic, siblings tend to be infected at a young age at different times. In rural areas, where epidemics occur, siblings are more likely to be infected concurrently and at higher ages. Using the median age of infection, Foster (1984) predicted CFRs for urban and rural areas of Nigeria of 6% and 2% respectively. In fact the opposite pattern is found and rates are higher in rural areas (Aaby 1988). The increased proportion of secondary cases in rural communities could explain this difference, though differences in health care facilities and living standards should also be considered.

An extreme pattern of exposure is seen in isolated communities where the whole population can be infected in a single epidemic with mortality rates around 20% (Morley 1969). The high mortality can be attributed to the lack of uninfected people left to look after the sick (Morley 1969), and to the relatively high death rates in adults (Peart & Nagler 1954), but intensive exposure due to simultaneous infection could contribute. Similarly high case fatality rates occur in refugee camps (Toole & Waldman 1990) and could be explained by intensive exposure as well as by the poor conditions.

For measles, Aaby has inferred differences in infecting dose from the observed difference in outcome between index and secondary cases. The results are consistent in studies carried out by different investigators in different places at different times and do not appear to be explained by confounding factors. It should be possible to use this distinction between index and secondary cases to investigate dose effects in other infections transmitted by the same route. Possible studies range from correlational studies comparing the proportion of secondary cases in an outbreak to a marker of severity of the outbreak (such as CFR), through case-control studies comparing exposure status of severe or not severe cases of disease, to community based studies.

Several studies of chickenpox have compared severity of disease between index and secondary cases. Ross (1962) found a larger average number of pox per child in secondary cases than primary cases among the controls in a trial of gamma-globulin. The secondary cases were younger and the results were only presented in broad age categories (6-59 months and 60-143

months). In recent trials of acyclovir treatment for chickenpox the case order in the family has been considered as a potential bias in the results and so has been recorded. Since the object of these papers was to assess the effect of acyclovir, the results relating to case order are incompletely presented. Dunkle et al (1991) studied 197 primary cases and 160 secondary and tertiary cases with a median age of 5, who received placebo. They reported that "the extent and duration of cutaneous disease were significantly greater in second or third cases in the same household than in first cases ($p < 0.001$), but the frequency and duration of fever did not differ". However, "in a regression model the maximum number of lesions could not be correlated with ... order of household occurrence". Balfour et al (1990), among 51 primary and 50 secondary cases with a median age of 7, randomized to receive acyclovir or placebo, found that secondary cases had more protracted disease. "They took significantly longer to begin to heal skin lesions ($p = 0.005$), to develop crusts ($p < 0.005$), and to experience cutaneous improvement ($p < 0.01$) in comparison with placebo-treated children who had primary cases." These results were not adjusted for age.

Less direct data are available that allow comparison of mortality rates of infections to measures of overcrowding. Crowded housing has been found to correlate with mortality rates in children from diphtheria, measles, tuberculosis and whooping cough (Payling Wright & Payling Wright 1942). However, these results are too remote from measurements of dose and severity to be useful: overcrowding is not the same as measures of secondary or even multiple cases; incidence rates are likely to be higher in overcrowded conditions, and even where case fatality rates are available or can be calculated, they will be influenced by factors related to overcrowding such as poverty and malnutrition. In Liverpool in the 1920s case fatality rates for diphtheria and scarlet fever decreased from the (crowded) central zone to the middle and outer zones (Stallybrass 1931). Children in the central zone acquired the diseases younger, and it is possible that less severe cases were under-reported in the central zone. In these data the proportion of secondary cases to primary cases was highest in the outer zone, emphasising that overcrowding does not necessarily correlate with a high proportion of secondary cases.

Amount of excretion by the index case

Transmission experiments with Group A streptococci in man found that acquisition rates increased with increasing quantities of streptococci isolated from the nearest carrier, and were higher in those exposed to nose carriers than throat carriers (Wannamaker 1954).

For measles, severe disease appears to lead to severe disease. In Kenya (Aaby & Leeuwenberg

1990) among children under 6 years, the CFR for secondary cases exposed to an index case who died was 3/11 (27%), compared to 20/329 (6%) for those exposed to an index case who survived (RR 4.69, 95% CI 1.64-13.41). This is consistent with intensity of exposure being a major factor if the assumption is made that fatal cases excrete more virus. It has been shown that children with severe measles excrete giant cells for longer (Schiefele & Forbes 1972). Giant cells contain viral particles, but in another study (Dossetor et al 1977) viral culture was negative after the acute phase. Alternatively, this relationship could be due to confounding: families where one child dies may be the most likely to experience another death for socio-economic or genetic reasons.

In Senegal (Garenne & Aaby 1990) the CFR increased with each generation of attack. The Sereer communities studied live in large compounds and as settlements are scattered have an epidemic pattern of measles. In larger compounds five or more generations of measles could be identified. Increasing CFRs from generation to generation were found, with odds ratios compared to the index cases, infected in the village, of 1.4 for the first generation of secondary cases, 2.4 for the second, 3.7 for the third, 5.5 for the fourth and 16.1 for the fifth and subsequent generations. The results were unchanged by controlling for the number of cases within the compound, so confounding by compound size is unlikely. If more severe cases secrete more virus this increase in severity could be explained by ever increasing exposure.

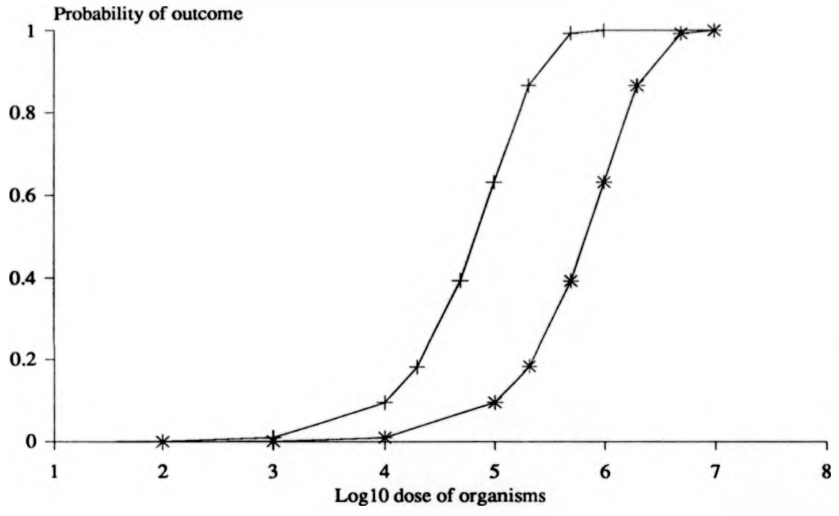
Family studies of tuberculosis in the USA in the 1930s and 1940s found higher attack rates when index cases were sputum positive than when they were negative (Putnam 1936, Brailey 1940, Puffer et al 1942, Stewart et al 1943). This could be interpreted in several ways. If the sputum negative cases excrete low doses it may be an example of a higher infecting dose giving higher attack rates, as expected. However it may simply indicate that a proportion of cases classified as sputum negative were or had been at some point sputum positive, or that some family members of sputum negative cases were exposed to tuberculosis elsewhere. If secondary cases in families of sputum negative cases are actually infected elsewhere, outside the household, then their infecting dose may be lower than that of the true secondary cases of sputum positive cases. It is therefore possible that, one way or another, those who become ill after exposure to a sputum positive case in the family are exposed to a higher dose than those ill after exposure to an apparently sputum negative case.

In prospective studies in Williamson County, Tennessee, index cases were classified as (1) fatal and manifest sputum positive and (2) other manifest and latent apical, and the experiences

of their household contacts were compared. Among coloured families (Puffer et al 1942) there were 22 deaths from 27 cases (81.5%) among the contacts of the first group and 2 deaths from 5 cases (40.0%) among contacts of the second, during the period of observation (RR 2.04, 95% CI 0.69-6.05). This difference may be due to the shorter period of follow up for the cases in the second group. Also, this group had a lower mortality rate from diseases other than tuberculosis so there may have been other differences between them and the first group. Among white families (Stewart et al 1943) the case fatality rates were lower and were similar in the two groups of contacts: 5/18 (27.8%) in the first and 5/17 (29.4%) in the second. The second group again had a lower mortality rate for non-tuberculosis deaths.

Figure 1.3.1. Probability of illness under the hypothesis of independent action, assuming uniform host resistance.

p = probability of outcome arising from each single organism
d = dose (number of organisms)
Equation of curves = $1 - (1 - p)^d$



Probability of outcome for different doses:

Dose	Left hand curve "disease" ($p = 10^{-5}$)	Right hand curve "severe disease" ($p = 10^{-6}$)
10^4	0.0952	0.00995
10^5	0.632	0.0952
10^6	0.99995	0.632

Intervention lowering dose from 10^5 to 10^4. Protective efficacy:
vs disease = 1 - 0.0952/0.632 = 84.9%
vs severe disease = 1 - 0.00995/0.0952 = 89.5%

Intervention lowering dose from 10^6 to 10^5. Protective efficacy:
vs disease = 1 - 0.632/0.99995 = 36.8%
vs severe disease = 1 - 0.0952/0.632 = 84.9%

SECTION 2

SALMONELLAE

INTRODUCTION

Some information is available on all three of the factors (host characteristics, pathogen characteristics, and factors affecting the interaction) that determine the outcome when a host is challenged with salmonellae. Different strains of mice have different susceptibilities to *S typhimurium* and this is dose-dependent. Selective breeding has shown that susceptibility is under genetic control, and three different genes have been identified (O'Brien et al 1980). As well as varying in susceptibility, different in-bred strains have different latent periods to death (Hormaeche 1975).

Salmonella infections are more severe at the extremes of life (Parker 1990) and in some mouse strains the males are more susceptible (Gowen 1960). Reinfection with typhoid can occur (Marmion et al 1953) but is thought to be rare (Hornick et al 1970). Vaccination provides some protection from typhoid, with estimates of vaccine efficacy around 50 - 75% for the more commonly used vaccines (Parker 1990). However, when infection occurs in vaccinated individuals the severity of the illness is unaltered (Hornick et al 1970). Other host factors may also be important, for example debility, malnutrition (Blaser & Newman 1982) and the taking of antibiotics (Hornick & Woodward 1966).

The presence of the Vi antigen has been shown to be an important determinant of virulence for typhoid and paratyphoid. Both Vi positive and Vi negative strains are ingested by human neutrophils but Vi positive strains manage not to activate the intracellular oxidative killing system of the leucocyte (Parker 1990). In volunteer experiments Vi positive strains produced a higher proportion of infected and symptomatic cases. It has also been found that different strains of *S typhi* produce different amounts of the Vi antigen (Old 1990). Among the other salmonellae, virulence is thought to depend on the lipopolysaccharide cell wall, the O antigens, and factors coded for by plasmids (Old 1990).

The pathogen-host interaction, is influenced by factors affecting the delivery of organisms - the vehicle and route of infection - as well as the infecting dose itself. The initial dose and the vehicle interact with host factors to influence the number of organisms reaching the intestine (Blaser & Newman 1982). High gastric pH and fast gastric transit times would both increase the proportion of the inoculum which survived passage through the stomach. Vehicles with a high buffering capacity would raise the gastric pH, but their effectiveness will depend on the host response, as was discussed for cholera (Music et al 1971). In experimental infections the

route of entry will be important, and aerosol transmission has been suggested in some salmonella outbreaks in infants.

While using the term "infecting dose" to mean the initial inoculum, it is the dose delivered to the intestines which will have the direct influence on outcome. Some of the variation in incubation period between individuals given the same dose may be due to the factors altering the number of bacteria that reach the intestine. Indirect measures of dose which involve the point of consumption of organisms - the amount of food consumed, the time when the food is eaten and the type of vehicle - all reflect the intial inoculum. The incubation period and attack rate reflect the dose in the intestine, as they depend on the host response. If dose does influence severity, measures which reflect the dose in the intestine would be expected to show a closer association with severity than measures reflecting the initial inoculum. (As mentioned in Section 1, confounding by strain and by host susceptibility will also tend to increase associations between incubation period or attack rate and severity.)

I chose the salmonellae for study because they are important pathogens and it has been suspected for some time that dose does influence severity (Miner 1922) but the evidence as quoted (eg Blaser & Newman 1982, Esrey et al 1985) is far from complete. The available published evidence has not previously been pulled together.

After a short review of the published evidence from experimental infections I review reported outbreaks of salmonella infections in the form of an analysis comparing severity by indirect measures of dose within and between outbreaks. Then I present an analysis of a single large outbreak and finally an analysis of data from volunteer experiments with typhoid from the Center for Vaccine Development, Baltimore.

To comply with University of London regulations I should like to point out that much of the material on salmonellae was included in my MSc thesis (MSc awarded January 1992). The MSc thesis contained a brief review of the animal literature, a descriptive review of published typhoid and salmonella epidemics, a study comparing published typhoid epidemics, a study comparing published salmonella epidemics and an analysis of a single salmonella outbreak.

For the PhD the literature was searched more thoroughly. The descriptive review contains more epidemics and the comparison of published typhoid epidemics has been extended from 52 to 69, so the analysis was re-done. For the single outbreak the analysis was also re-done after incorporating some further data from the original questionaires. All sections have been extensively rewritten and the analysis of experimental typhoid data has been added.

PUBLISHED EXPERIMENTAL EVIDENCE

When human volunteers were infected with *S typhi*, it was found that the attack rate increased and the median incubation decreased with increasing challenge doses (Table 2.2.1). The authors stated that there was no association between dose and severity of symptoms but no details were given (Hornick et al 1970). I have not found other published experiments in which human volunteers have been given different doses of typhoid.

McCullough and Eisele (1951a-d) gave varying doses of several non-typhoid salmonellae to volunteers in experiments designed to test pathogenicity and to determine the minimal infective dose. As the dose increased, the proportion with positive faecal cultures and, at higher doses, with clinical disease both increased, but there were too few ill volunteers with each strain for any further conclusions on dose and severity to be drawn, and interpretation is complicated by the use of many volunteers for more than one feeding.

Small animal experiments with salmonellae have been dominated by experiments testing models of microbial infection (eg Meynell & Stocker 1957) and, later, examining immunity and the genetics of host resistance (eg Hormaeche 1975). The models themselves are examined in the final Discussion (Section 4). In this section those experiments which give information on dose and severity will be discussed.

In a series of experiments, giving *S typhimurium* by intraperitoneal injection to mice, Meynell and Meynell (1958) found that the geometric mean time to death was inversely, linearly related to the logarithm of the dose of bacteria, at least for doses above the LD50. In some of the experiments where doses less than the LD50 were used there appeared to be a flattening of the curve so that the latent period to death was less clearly dependent on dose. This was interpreted as meaning that fatal infections at low doses are initiated by only one organism, but the confidence intervals were wide. Furthermore, by measuring the bacterial load in mice killed at various stages after inoculation, and in dead mice, it appeared that the whole inoculum multiplied exponentially until a constant critical load was reached and the mouse died.

The relationship between dose (above the LD50) and latent period to death has been shown consistently by other workers using different strains of mice and different salmonellae (eg Schutze et al 1936, Robson & Vas 1972, Hormaeche 1975). The more recent experiments were designed to study the genetics of resistance, so inbred strains of mice were used. The pattern

for doses less than the LD50 remains unclear, partly because large numbers of mice are needed in order to get sufficient dead mice for latent periods to be calculated. In Hormaeche's results (1975) the numbers in each group are small: four experiments using doses around or less than the LD50 suggest a levelling off of the log-dose/latent period curve, whereas three others do not. An inverse relationship between dose and time to death has also been found for chicks, using intravenous *S stanley* (Rao & Chauhan 1987) and in some but not all experiments with intraperitoneal *S typhimurium* (Milner & Shaffer 1952, Shaffer et al 1957).

Experiments on the multiplication of salmonellae *in vivo* by measuring counts in the liver and spleen of mice killed at intervals after inoculation have shown that the growth rate depends on the virulence of the organism (as determined by the LD50, the proportion of mice dying, or the mean time to death) (Collins 1969) and on the strain of the mouse (Hormaeche 1975). The growth rate was found to be constant over a range of challenge doses but direct measures were not made for small doses in the early stages of infection (Collins 1969). Examination of the curves given in Collins' papers suggests that the growth rate may be greater after large doses in the early stages (Collins 1972a,b), and certainly infections by different routes show different initial growth rates before the "constant" phase is reached (Collins 1969). In one of Hormaeche's experiments the time to death was disproportionately shorter for the very high doses suggesting an increase in growth rate (Hormaeche 1975) but this was not seen in Meynell's experiments (Meynell & Meynell 1958). Infections with low doses which would not have been fatal show an initial rise in bacterial counts followed by a fall (Meynell & Meynell 1958, Collins 1972b).

Even if the growth rate is constant, it is compatible with a dose-severity relationship, since the point from which the curve starts its rise varies with the initial dose (Hormaeche 1975, Meynell & Meynell 1958). This correlates with the inverse relationship between dose and time to death. Low doses allow more time for mobilization of host defences before lethal numbers of organisms are reached, and it might be expected that the defences would be more effective against smaller loads.

The time to death reflects the incubation period as well as any differences in length of symptomatic illness, so the relationship between dose and time is expected. The report of one of Collins' experiments with specific pathogen-free mice given oral *S enteritidis* mentioned that all 10 receiving 2×10^2 organisms survived, although half of them had "systemic infections", whereas among the 20 given 2×10^3 or 2×10^4 organisms there were "many deaths" (Collins

1972a). Whether the "systemic infection" was clinically apparent or not is not clear. In small animals it is not easy to measure levels of severity of illness short of death, but in one study of chickens the duration of diarrhoea was found to increase after higher doses of *S enteritidis* (Humphrey et al 1991).

With calves, morbidity can be assessed more easily and there is some evidence of a dose-severity relationship in experiments which were designed to study pathogenesis. Unfortunately, since calves are expensive, the numbers used tend to be small. DeJong and Ekdahl (1965) gave three different doses of *S typhimurium* to 12 calves either orally, in milk, or conjunctivally. After 10^{11} organisms 3/4 died. All 4 who received 10^{8} were ill but recovered, and the 4 who received 10^{6} had positive faeces but were asymptomatic. Using the same strain of *S typhimurium*, Robinson and Loken (1968) found more prolonged excretion of bacteria after larger oral doses in asymptomatic Jersey calves at two different ages.

Wray and Sojka (1978) gave *S typhimurium* phage type 1 (var 5) orally in a nutrient broth to Jersey calves. The numbers were small, especially in the low dose groups. Their results suggest an increased case fatality rate and more prolonged symptoms at higher doses. (Their measurements of duration of signs and of excretion are difficult to interpret as they are determined both by recovery and death.)

Smith and Jones (1967) gave *S Dublin* in two different doses to a mixture of Ayreshire and Jersey calves. Sixteen received 10^{10} organisms; all died and the mean time to death was 4.1 days (SE = 0.4). Six received 10^{7} organisms; all had signs of illness, and one died on the seventh day.

Typhoid occurs naturally only in man and there is no good animal model (Hornick et al 1970). Using large doses, a typhoid-like illness can be induced in chimpanzees. Experiments using chimpanzees use very small numbers and I have found only one study in which different doses were given to more than one animal per group (Edsall et al 1960). The bacteria were inoculated into bananas which were then given to the chimpanzees. The attack rate increased with dose and it was noted that "no clear cut obvious connection between challenge dose, immune response and the duration of the carrier state could be observed".

Table 2.2.1. Relationship of dosage of *S typhi*, Quailes strain to disease (from Hornick et al 1970)

Dose (no. of organisms)	No. of volunteers	No. with disease (%)	Incubation period (days)	
			Median	Range
10^9	42	40 (95%)	5	3 - 32
10^8	9	8 (89%)		
10^7	32	16 (50%)	7.5	4 - 56
10^5	116	32 (28%)	9	6 - 33
10^3	14	0 (0%)		

ANALYSIS OF REPORTED OUTBREAKS OF SALMONELLA INFECTIONS

The indirect measures of dose which can be used in assessing natural infections were discussed in Section 1. Information on relationships between these measures of dose and outcome in reports of natural infections with salmonellae is very limited and I have therefore also made comparisons between outbreaks. Within outbreaks I have used four proxy measures of dose: amount of food, the time when food is eaten, attack rate and incubation period. Between outbreaks I have used three measures: type of vehicle, incubation period and attack rate.

The validity of the results depends on how well the proxy measures reflect the true dose. In the volunteer experiments reviewed above, dose was related to attack rate (Hornick et al 1970, McCullough & Eisele 1951a-d) and incubation period (Hornick et al 1970). These associations have also been found in natural outbreaks. Miner (1922) noted that the mean incubation times of three water-borne typhoid epidemics were about twice as long as those of two food-borne epidemics and thought that this was probably due to differences in dose. In a typhoid outbreak at a school picnic, 23 people became ill after eating contaminated chocolate icecream (Cumming 1917). Those who ate a mixture of types of icecream (and therefore probably less chocolate icecream) had longer incubation periods (mean 9.5 days compared to 6.1 days, $p < 0.001$ Wilcoxon). In a large water-borne typhoid outbreak in Zermatt, information was presented by area according to the water supply (Bernard 1965). As the contaminated water became progressively diluted by other supplies, the attack rate by area decreased and the median incubation period increased. An outbreak of *S enteritidis* following a wedding in Connecticut affected 171 people (Mintz et al 1991). Those who ate more of the contaminated Hollandaise sauce had shorter incubation periods. However, in a large outbreak of *S typhimurium* the attack rate was no higher in those who ate more than one piece of the chicken vehicle (Palmer et al 1990).

Blaser and Newman (1982) and Naylor (1983) draw attention to the apparent inverse correlation between attack rates and incubation periods for different typhoid outbreaks. These could be due to the common effects of dose, though variation in strain virulence between epidemics would give a similar result. Blaser and Newman (1982) presented data from 8 typhoid epidemics where information was available on both median incubation period and attack rate. Analysis of their data (after excluding one epidemic where the incubation period is based on just two people, and correcting the attack rate for the Mon Ark epidemic) shows a non-significant linear correlation ($r = -0.63$, $b = -0.139$, $t = 1.81$, $p > 0.1$). Naylor (1983)

presented similar data using the mode. He listed 7 epidemics wih sufficient information, 5 of which were included by Blaser and Newman and one of which has an attack rate of 100% based on the figures of only a subgroup of the people involved.

METHODS

I have used the proxy measures of dose in assessing whether dose influences severity of disease. Whilst varied measures of severity are used for within-epidemic analyses, for comparisons between epidemics the case fatality rate is used for typhoid and hospitalization rate for other salmonellae, for reasons discussed below.

Published reports were identified that gave data on severity and on a proxy measure of dose. They were found by searching the *Bulletin of Hygiene*, later *Abstracts of Hygiene* (from 1926), *The Lancet* (1920-45), *The American Journal of Hygiene* (1921-64), the British *Local Government Board, Medical Officer's Report* (later the *Reports to the Local Government Board on Public Health and Medical Subjects* and then the *Ministry of Health Reports on Public Health and Medical Subjects*, from 1900), the National Communicable Diseases Center, later the Center for Disease Control *Salmonella Surveillance Reports* (1964-76) and the *Morbidity and Mortality Weekly Reports* (from 1976): any references from these journals, or from already identified articles, which it was thought might contain sufficient data were followed up. For the analysis of single epidemics, all identified reports that gave data on severity and on a proxy measure of dose were included. Where the data were anecdotal the reports are only mentioned briefly, as there is likely to be a bias towards inclusion of positive findings in a report.

For comparison between epidemics, the selection criteria for typhoid and for the other salmonellae differ because of the necessary use of different proxy measures of severity. Within the criteria, all located published outbreaks were included.

For typhoid, case fatality rate was chosen as an outcome measure which could be extracted from reports and could be compared between epidemics. As case fatality rates are much lower with antibiotic therapy, outbreaks occurring after 1945 were excluded. Common source typhoid epidemics were identified in the published literature and included in the study if they contained sufficient information on case fatality rate and attack rate or incubation period and involved at least eight cases. Outbreaks among hospital patients were excluded. As information on

incubation period was scarce, post-war epidemics where the incubation period was given were also identified. They have only been included in the analysis when case fatality rate is not being considered.

Correlations were sought between: attack rate (AR) and incubation period; attack rate and case fatality rate (CFR); and incubation period and case fatality rate. For reasons addressed in the discussion, the initial analysis was carried out unweighted (each epidemic carrying equal weight regardless of size). As an attempt to separate epidemics with more accurate information, a subgroup of pre-war epidemics was identified where the population exposed was well defined (such as guests at a reception, or people supplied with milk from one farm). For this subgroup weighted analysis was done using logistic regression. The models used were:

$$\text{Logit AR} = \text{Constant} + \beta(\text{Incubation})$$

$$\text{Logit CFR} = \text{Constant} + \beta'(\text{Incubation})$$

$$\text{Logit CFR} = \text{Constant} + \beta''(\text{AR})$$

For the food-poisoning salmonellae I have again used attack rates and incubation periods as measures of dose. Fatalities from non-typhoid salmonellae are unusual, and descriptions of cases are not detailed enough for any symptom-based measure of morbidity to be used. The only readily available measure of severity is the number of cases requiring hospitalization. Since this is obviously time- and culture-dependent I have only used epidemics reported in the National Communicable Disease Center (later Center for Disease Control) *Salmonella Surveillance Reports*. The hospitalization rate was taken as the proportion of cases who were hospitalized. Surveillance reports of common source outbreaks from the period 1964 to 1974 were included if they contained sufficient information and involved more than eight people. Outbreaks involving hospital patients or mixed infections were excluded.

Correlations were sought between: attack rate and hospitalization rate (HR); median incubation period and hospitalization rate; and attack rate and median incubation period. The analysis was carried out using unweighted linear regression and logistic regression. The models used for the logistic regression are:

$$\text{Logit HR} = \text{constant} + \beta(\text{AR})$$

$$\text{Logit HR} = \text{constant} + \beta'(\text{incubation})$$

RESULTS

Analyses of Single Epidemics

Amount of food

For typhoid only two reports allow comparison of amount of food with outcome. In the outbreak following a school picnic the case fatality rate was 3/17 for those who had whole portions of the affected ice cream and 0/6 for those with half portions (p = 0.5 Fishers 2-tailed test) (Cumming 1917). The other report concerned a milk-borne outbreak involving 68 cases (Sirois 1942). The author noted that among those who were ill, those who only took milk in their tea or coffee had very mild attacks.

For the food-poisoning salmonellae I have found 11 reports which provide information on amount of food and severity. Mintz et al (1991) provide the most detailed breakdown of outcome by amount of food consumed, in an outbreak of 171 cases of *S enteritidis* infection due to contaminated Hollandaise sauce. As the amount of sauce used increased, there were increases in the proportion of cases with body aches, nausea and vomiting, the maximum number of stools passed per 24 hours, and acute weight loss, but not in duration of illness. Taylor et al (1984) describes a small family outbreak of *S typhimurium* in which the person who had eaten the most of the affected vehicle died. In five other reports, although it is stated that those who ate more had more severe disease, no supporting evidence is given (Balice 1958, Bierschenck 1962, De Blasi & Scotti 1950, Harding 1966, Gomez Lus & Gimenez Martinez 1965).

Four reports failed to find an association. In an *S typhimurium* outbreak affecting nearly 200 people, there were no differences in the mean maximum stool frequency or in the duration of illness for those who ate one or more pieces of the chicken vehicle (Palmer et al 1990). However, the attack rate was not related to the number of pieces of chicken eaten either, so the bacteria may have been unevenly distributed. (This outbreak is the subject of Section 2.4.) Two other negative reports refer to meat pies. In the first the pies were baked in two separate lots on different days, so uniform contamination is unlikely (Miller et al 1955). In the second, most of those who had severe disease had eaten the smaller pies but had kept them unrefrigerated for 24 hours (Brockbank et al 1950). The last report refers to only six cases (D'Aoust 1985).

The time when food is eaten

In addition to the meat pie outbreak (Brockbank et al 1950), five anecdotal reports of food-poisoning salmonellae suggest that food eaten later (after allowing time for bacteria to multiply) gave rise to more severe disease (De Blasi & Scotti 1950, Cook & de Costabadie 1947, Semple et al 1968, Anonymous 1988, Möller 1955).

Attack rate and Outcome

In an extended water-borne typhoid outbreak in Bolton-upon-Dearne in 1921, attack rates and case fatality rates were reported by district, and showed no particular pattern (Shaw 1922). I have found no other studies of salmonella where attack rate and measures of severity are given by area.

Incubation period and Outcome

In five reports of typhoid outbreaks individual incubation periods can be related to outcome (Cumming 1917, Desranleau 1946, Lumsden 1912, Bulstrode 1902-3, Mollohan & Reid 1967). In some, including the two larger outbreaks (Cumming 1917, Desranleau 1946), the proportion who died was larger among those with shorter incubation periods, but in none of the epidemics did the differences approach significance at the 5% level.

For the food-poisoning salmonellae the picture is rather different. Of nine reports which give sufficient details, only one (Horwitz et al 1977) failed to find an association between incubation period and severity. In an outbreak of *S newport* food-poisoning in Sweden (Bille et al 1964) information was available on 161 people; those with shorter incubation periods had more severe illnesses. In a large outbreak of *S thompson* food-poisoning in Tennessee onset times were on average earlier in the 51 who were hospitalized than in 72 others (Fowinkle et al 1970). Balice (1958) describes a severe outbreak of *S typhimurium* in Italy. All 83 people who ate the affected food became ill and there were five deaths. The mean incubation period overall was 21 hours (range 8 to 30 hours), and for the five who died it was 14 hours. However, in an outbreak due to *S newport* where information was available for 105 cases, it was noted that the median incubation period was 29 hours overall, and 30 hours for those with "severe illness", defined by the number of different symptoms experienced. The number of cases in this group is not stated. It was also stated that there was no relationship between the length of the incubation period and the duration of illness (Horwitz et al 1977). The other five reports all suggest an association, but are based on small numbers of cases or have small numbers of deaths as their only outcome measure (Miller et al 1955, Janeway et al 1971,

Holmes et al 1967, Anonymous 1988, Hauser 1945).

Comparisons between Epidemics: Typhoid

Sixty-nine typhoid epidemics fulfilled the criteria, including eight post-war epidemics (see Appendix 1). Most were from Britain or the United States. Thirty-five were water-borne. Incubation periods were available for 27 epidemics, including 19 pre-1945 epidemics. The median incubation period was used whenever it was given. Attack rates were available for all but four of the epidemics.

The distribution of attack rates, incubation periods and case fatality rates are shown in Figure 2.3.1. The associations between them are shown in Figures 2.3.2-3. A log scale is used for the attack rate as the distribution of attack rates is skewed to the right. High attack rates were associated with short incubation periods but no significant correlations with case fatality rate were found.

Water-borne epidemics had longer incubation periods and lower attack rates. There was no difference in case fatality rates between water- and food-borne epidemics (Table 2.3.1). When the vehicle (water or food) was added to the regression equations, in a multiple regression model, the correlation between incubation period and attack rate was no longer significant, and the regression coefficient was reduced from -4.0 to -2.8 (95% CI -6.1 to 0.5). There was still no association between case fatality rate and either attack rate or incubation period.

The analysis was repeated for circumscribed pre-war epidemics where the population exposed was well defined. Thirty-one epidemics fulfilled this criterion. No associations between incubation period, attack rate and case fatality rate were found in the unweighted analysis. Logistic regression analysis for this subgroup of circumscribed epidemics showed a negative association between incubation and attack rate based on 11 epidemics which were all food-borne (likelihood ratio statistic (LRS) 93.2, one degree of freedom (df), $p < 0.001$, proportion of deviance explained = 15%). Incubation period showed a just significant positive association with case fatality rate based on 13 epidemics (LRS 4.4, 1 df, $p = 0.04$). There was no association between attack rate and case fatality rate.

There was a non-significant negative correlation between case fatality rate and the date of the epidemic. When the year of the outbreak was included in multiple regression or logistic

regression models between case fatality rate and incubation period or attack rate the regression coefficients hardly changed.

Comparisons between Epidemics: Food-Poisoning Salmonellae

Sufficient numbers of suitable reports were available for analysis for four different salmonellae: *S typhimurium*, *S enteritidis*, *S thompson*, *S infantis*. Details of the epidemics are given in Appendix 2. All except one epidemic (of *S typhimurium*) were food-borne. Plots of hospitalization rates by attack rates are shown in Figure 2.3.4.

S typhimurium

Information was available for 16 epidemics. Linear regression showed no relationship between attack rate and hospitalization rate but logistic regression showed a positive correlation, giving the model:

$$\text{Logit HR} = -5.6 + 0.0406(\text{AR})$$

$$\text{ie} \quad \text{HR} = \frac{e^{(-5.6 + 0.0406 \times \text{AR})}}{1 + e^{(-5.6 + 0.0406 \times \text{AR})}}$$

This model explained 58.7% of the deviance. When the one large water-borne epidemic was excluded, the coefficient decreased to 0.0201 (SE 0.0058) but the model remained highly significant: LRS =12.5, 1 df, $p < 0.001$.

S enteritidis

The linear regression, based on 11 epidemics, did not show a significant association, but again logistic regression showed a positive correlation, giving the model below, which explained 10.7% of the deviance:

$$\text{Logit HR} = -3.5 + 0.0223(\text{AR})$$

After excluding the one outlying epidemic with a high hospitalization rate (Figure 2.3.4b) the association was lost.

S infantis

The linear regression model, based on seven epidemics was just significant, but is influenced by a small epidemic with high attack and hospitalization rates. ($r = 0.75$, $b = 0.71$ (95% CI 0.16-1.25), $a = -7.96$, $t = 2.55$, $p = 0.05$.) Logistic regression also showed a positive correlation, giving the model:

$$\text{Logit HR} = -3.6 + 0.0415\ (\text{AR})$$

This explained 43.6% of the deviance. After excluding the small epidemic with the high attack and hospitalization rates, the coefficient in the logistic regression model decreased to 0.0237 (SE 0.0094) and the strength of the association was reduced, but it remained significant (LRS = 6.2, 1 df, p = 0.01).

S thompson

The scatter plot (based on eight epidemics) shows no trend in the results, and neither linear nor logistic regression models had coefficients which were significantly different from zero.

Incubation periods were only available for a few epidemics of each serotype. For *S typhimurium*, six epidemics contained information on both incubation and hospitalization rate: no association was found using linear or logistic regression. Even less information was available for the other serotypes.

DISCUSSION

For typhoid, the results confirm the suspected relationship between incubation period and attack rate. Since there is no evidence that more virulent forms of typhoid are found in food than in water, the long incubation periods and low attack rates found in water-borne epidemics suggest that the dose in these epidemics is, on average, smaller. By contrast, correlations between attack rate or incubation period and case fatality rates were not found (with one exception), even though both dose effects and any differences in virulence would be expected to lead to such correlations.

There are, however, limitations to the typhoid data. The attack rate depends on both full ascertainment of cases and correct ascertainment of those at risk, here taken as those exposed during the epidemic. Immune status was usually unknown. The most accurate estimates of those exposed are those obtained from circumscribed epidemics occurring after specific meals or at a camp where everyone can be traced and those who consumed a particular food or drank the water can be identified. Milk-borne epidemics where the numbers of people on the milk round is known give reasonable figures. Where the domestic water supply is the source, the number of people using the supply is only an approximate estimate, and those who avoid being truly exposed by buying or boiling their drinking water is unknown. Also, it is unlikely that the whole of the supply is significantly contaminated. (In the water-borne typhoid epidemic in

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Zermatt, attack rates by area varied from less than 1% to over 25%, suggesting considerable variation in dose (Bernard 1965).) Full ascertainment of cases is also difficult, but again it is likely to be most complete for the most circumscribed epidemics where individuals are actively traced. Large epidemics rely on notification of cases which will be incomplete.

The incubation period can be estimated only for point source epidemics and then only if dates of onset of illness rather than dates of notification are available. Late cases may be missed and secondary cases may be mistakenly included (leading to under- and over-estimation of the median incubation period respectively). The median incubation has been used since the epidemic curve is approximately log-normal, and it is usually the measure quoted. For a few epidemics where only the mode or "average" was given, that was used instead; they are usually similar (see Appendix 1).

Identification of deaths from typhoid is probably more complete than identification of cases, leading to overestimation of the case fatality rate, to an extent which will vary from epidemic to epidemic depending on completeness of case ascertainment. It is a crude measure of severity, particularly as the numbers are small in some of the epidemics, and unmeasurable factors associated with the place of the outbreak would be expected to influence how many die.

Too few of the epidemics give sufficient information on the age, sex, or immune status of the people involved for these variables to be taken into account. The year of the epidemic may be expected to be associated with the case fatality rate, but would only be associated with incubation period or attack rate if there was a change in the predominant strains or if methods of investigating or reporting the outbreaks changed. Controlling for the year of the epidemic did not affect the results in the multiple regression analyses.

Epidemics could only be included in the study if they contained sufficient information. All identified epidemics which fulfilled the criteria were included, but they are not necessarily representative of all typhoid epidemics.

Although some of the data problems will lead to non-differential misclassification (and therefore to underestimation of associations) certain directions of bias appear likely: attack rates are likely to be unduly low in water-borne outbreaks due to both underestimation of cases and overestimation of susceptibles, and case fatality rates will be disproportionately high in epidemics with poor case ascertainment, which includes most of the water-borne epidemics. It

was felt that the large epidemics were often more inaccurate and more likely to be biased than the smaller epidemics so calculations are presented both weighted and unweighted.

Turning to the data, the finding of the expected correlation between attack rate and incubation period suggests that the data are not so crude as to be useless. Similarly, for water-borne epidemics, although the low attack rates may be due to bias, the longer incubation periods are unlikely to be, and the finding fits with the expected low dose of organisms in water.

The case fatality rates were the same in water as in food-borne epidemics. This could be true, reflecting no dose-effect, or could be due to bias giving falsely high case fatality rates in water-borne outbreaks. Attack rates did not predict case fatality rates. This could be true or could reflect the falsely low attack rates and high case fatality rates of less well investigated outbreaks. No association was found when consideration was restricted to circumscribed outbreaks. Incubation period did not predict case fatality rate in most of the analyses. Again, although this may be true both variables, and particularly case fatality rate, are subject to considerable error in measurement. The weighted analysis of the circumscribed epidemics produced a surprising positive correlation between incubation period and case fatality rate. This was only just significant.

Overall, the comparison of data between typhoid epidemics provides no evidence of an association between dose (as measured by incubation period, attack rate or vehicle) and severity (as measured by the case fatality rate). While the data are too crude for an association to be excluded, this finding contrasts with the outbreaks due to other salmonellae but fits with the conclusions from Hornick et al's volunteer studies (1970).

The results from food-poisoning salmonellae provide evidence of a correlation between attack rates and case-hospitalization rates. The evidence for incubation period was too rudimentary to be useful.

For the non-typhoid salmonellae the unweighted linear regression models are too simplistic as the epidemics range in size from 10 cases to several hundred (and to 14000 in the one water-borne epidemic - see Appendix 2). Unlike the typhoid data, and with the possible exception of the water-borne epidemic, there is no reason to believe that the figures in the larger epidemics are any less accurate than those in the smaller epidemics, so it seems appropriate to weight the epidemics according to size. The logistic regression model has the added advantage that it does

not require the variables to be normally distributed, which is more appropriate given the small numbers involved. For three of the serotypes of salmonella the coefficients were highly significantly different from zero, and for *S typhimurium* and *S infantis* around 50% of the deviance in the results was explained by the model.

The epidemics studied here are not necessarily representative of all epidemics in the USA as not all are reported, and only the reports containing sufficient information could be included. Although all of the epidemics came from one country over a short period, criteria for hospitalization may have varied between epidemics, probably leading to under-estimation of any correlation. However, the hospitalization threshold may be higher in a large epidemic, which would bias the results in the weighted analyses. Lack of data prevented controlling for age, though those at the extremes of age are more likely to be hospitalized and may also have different attack rates.

The results for food-poisoning salmonellae point to a positive correlation between attack rate and hospitalization rate at least for some types of salmonella. This is consistent with a dose effect whereby higher doses give higher attack rates and more severe disease, though differing virulence between different strains would give similar results. The results from the single epidemics tend to support the dose-severity correlation, though within an epidemic a correlation between incubation period and severity could be due to individual differences in susceptibility as well as dose.

Table 2.3.1. Comparisons between food and water-borne typhoid epidemics

	Vehicle	No.	Mean (95% CI)	*p* (t-test)
Incubation period (days)	Water	8	18.5 (15.2-21.8)	
	Food	18	13.2 (11.0-15.4)	0.007
Attack rate (%)[1]	Water	34	3.8 (2.3-6.3)	
	Food	28	16.7 (10.8-25.9)	< 0.001
Case fatality rate (%)[2]	Water	32	10.3 (8.4-12.2)	
	Food	26	11.4 (8.0-14.9)	

[1] geometric mean given

[2] prewar epidemics only

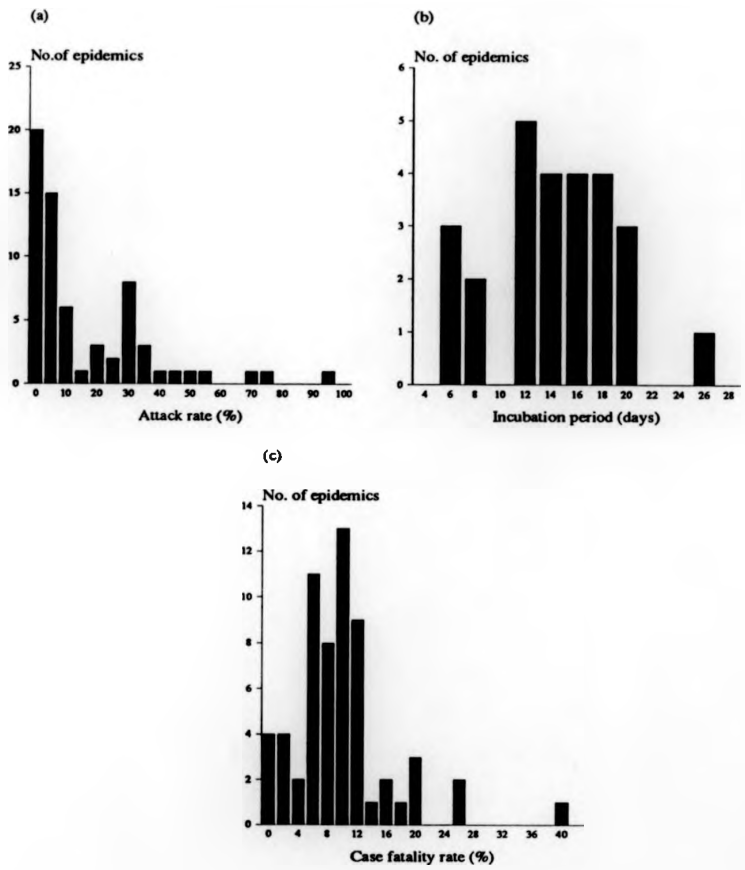
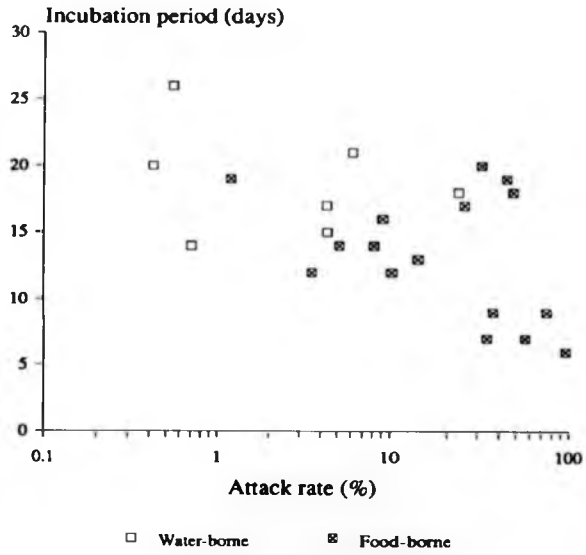


Figure 2.3.1. Typhoid epidemics used in the comparison study. (a) Attack rates for 65 epidemics. (b) Incubation periods for 26 epidemics. (c) Case fatality rates for 61 pre 1945 epidemics.



r = -0.55 (95% CI -0.78 to -0.17)
b = -4.01 (95% CI -6.64 to -1.38)
a = 18.92, t = 2.99, p < 0.01

Figure 2.3.2. The relationship between attack rate and incubation period for 23 typhoid epidemics. Each square represents an epidemic.

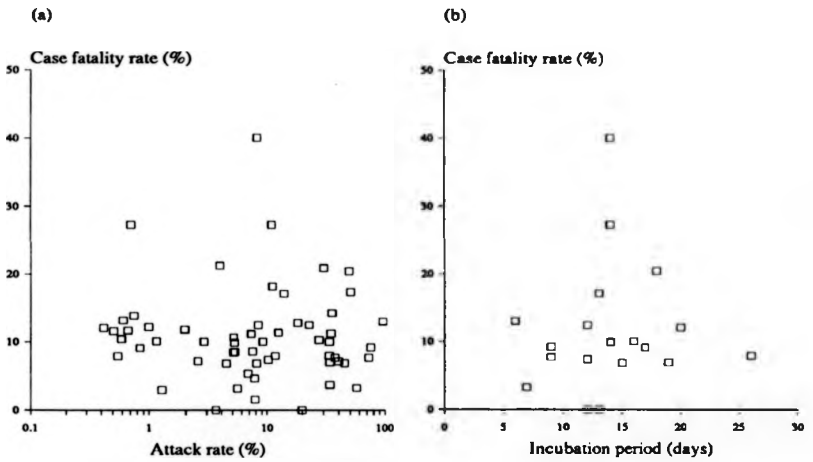


Figure 2.3.3. The relationship between typhoid case fatality rate and (a) attack rate (for 58 pre-1945 epidemics) and (b) incubation period (for 19 pre-1945 epidemics). Each square represents an epidemic.

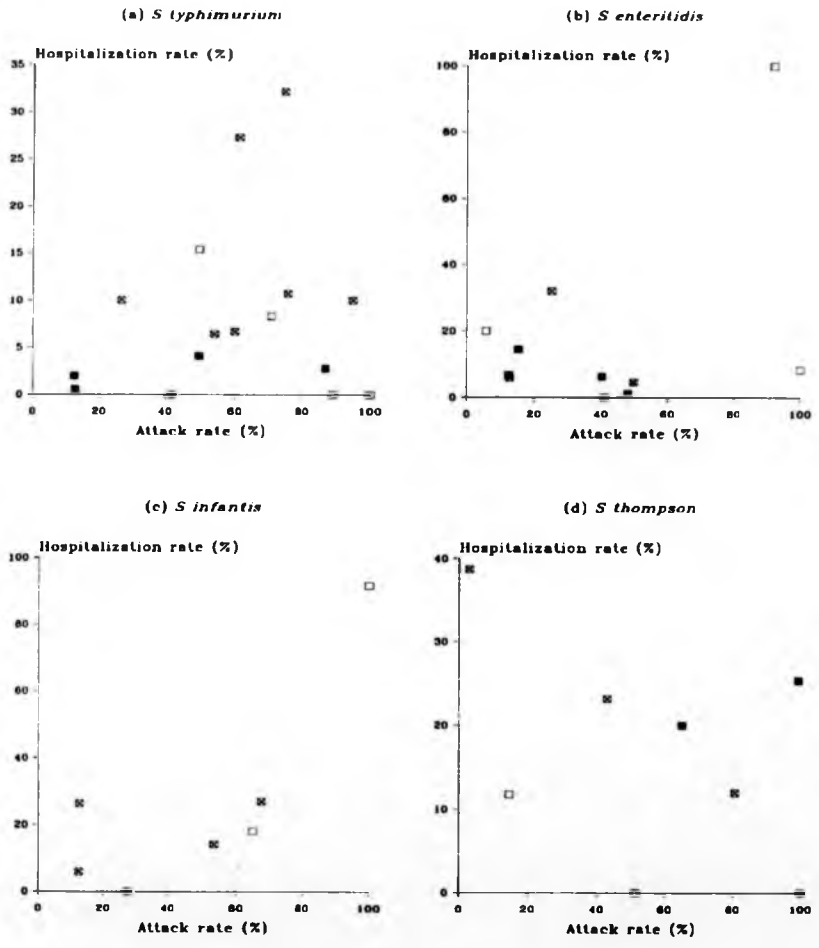


Figure 2.3.4. The relationship between hospitalization rate and attack rate for four food-poisoning salmonellae. Each square represents an epidemic. □ = epidemics of less than 20 cases; ■ = epidemics of 20 - 100 cases; ■ = epidemics of over 100 cases.

ANALYSIS OF A *SALMONELLA TYPHIMURIUM* OUTBREAK

In this study I have investigated the associations between dose, incubation period and severity within a single large point source outbreak of *Salmonella typhimurium.*

In September 1986 an outbreak of *Salmonella typhimurium* affected delegates at a medical conference in Wales. The source of the infection was traced to chicken pieces served at a buffet lunch. Of 427 delegates at the conference, 360 (84%) returned questionnaires. Of these, 265 attended the buffet lunch and 195 became ill. The initial questionnaires were sent out four days after the outbreak, and follow-up questionnaires including questions on duration of illness and time off work were sent out about eight weeks later. The results of the outbreak investigation have already been published, and it was noted that those who had more than one piece of chicken had a similar attack rate to those who had only eaten one. Among those who became ill the authors noted that there was no difference in the mean maximum stool frequency or duration of illness in those under 45 years old who ate one or more pieces of chicken and that only one of those who had eaten two pieces of chicken reported paraesthesia (Palmer et al 1990). I have examined the associations with chicken consumption in more detail, including the association between chicken consumption and incubation period, and have used the incubation period as a second proxy marker of infecting dose.

MATERIALS AND METHODS

The data from the outbreak were available to me in the form of an Epi Info data file. I was able to fill in some gaps in the data from the original questionnaires. The incubation period was taken as the time from serving the buffet to the onset of the first major symptoms (diarrhoea, vomiting, fever or abdominal pain). Four of the 195 cases were excluded because no onset dates or times were recorded (two) or because of inconsistencies in the dates given (two). Where only the date was recorded (12 people) the time was taken as the median onset time for that day.

Several different measures of severity of disease were available. The maximum stool frequency per 24 hour period, the maximum temperature reached, the duration of symptoms and the time off work were collected as quantitative variables. Whether the person was admitted to hospital and the presence or absence of a range of symptoms were recorded as binary variables. I have

used all these measures. Information was also available on possible confounders: age, sex, prior antibiotic or antacid use, previous upper gastrointestinal tract surgery, achlorhydria, gastritis, peptic ulcer disease and immunosuppression.

Some of the questionnaires were incomplete and in presenting the results those with missing values for a particular variable were excluded. In each case I have stated on how many people a result is based.

When examining the data for correlations and fitting regressions, histograms were examined individually to see if they were normally or otherwise distributed (for example log normal). Scatter plots were compared on linear and log scales. On the basis of the histograms and the scatter plots, linear or log scales were chosen for the different variables, and a regression was fitted. The residuals from the regressions were examined to see if there was any remaining pattern or whether the model chosen explained all of the association.

RESULTS

All the results refer only to the 191 ill people for whom incubation periods are available. Of the 130 people who recorded their sex, 38 were female. Ages were available for 173 people and ranged from 21 to 61 years (mean 37). Table 2.4.1 shows the proportions of those ill who suffered from different symptoms. Thirty-two (of 175 who responded to this question) recorded that they were admitted to hospital.

Figures 2.4.1 and 2.4.2 show the distributions of incubation period, and of four measures of severity: the maximum frequency of diarrhoea stools; the maximum temperature reached; the duration of symptoms; and the time off work. The maximum temperature reached was felt to approximate to a normal distribution, and the others to log normal distributions. This was confirmed by examination of normal probability plots.

Incubation period was negatively associated with all of the markers of severity (Figures 2.4.3-6). Regression lines were fitted after log transformation of those variables which were felt to be log normally distributed. The correlation and regression coefficients are given with each figure. Plots of the residuals showed no remaining patterns.

Age and sex were considered as possible confounders. There was no association between age and incubation period. Among the measures of severity, age was weakly associated with maximum stool frequency but not with any of the others. Incubation periods were almost identical between the sexes and there were no significant differences between the means (or geometric means) of any of the measures of severity. Adding age or sex to the regression equations between incubation period and the measures of severity had little effect on either the significance of the associations found or on the regression coefficients.

Eight people who became ill had taken prior antibiotics or antacids. One of these had had previous upper gastrointestinal tract surgery. One additional person was receiving immunosuppressive treatment. None reported achlorhydria. This group of patients may be considered as being particularly susceptible to infection. Patients in this group, considered either by subgroup or together as "susceptibles", had geometric mean incubation periods and mean or geometric mean measures of severity which were similar to those for the other patients. Adding "susceptibility" to the regression equations hardly affected the regression coefficients. One patient reported gastritis, and one reported peptic ulceration without antacid use.

To see if incubation period predicted the outcome in terms of the symptoms suffered, the incubation periods were divided into tertiles and the proportion of patients suffering from each symptom, in each group was recorded. The results are shown in Table 2.4.2. As the numbers in some of the cells are small the validity of the results was confirmed using logistic regression. For most of the symptoms the proportion of people suffering from them increased as incubation period decreased. The proportion of people hospitalized also increased with shorter incubation periods (Table 2.4.2).

Younger people were more likely to vomit, have abdominal pain and headaches and be admitted to hospital, but were less likely to have diarrhoea. Women were more likely to vomit but there were no other differences between the sexes. Using multiple logistic regression, there was no evidence of confounding by age or sex in any of the associations with incubation period.

Twenty eight people ate two or more pieces of chicken. The geometric mean incubation period for those who ate two pieces was shorter than that for those who only ate one piece: 16.6 hours (95 % CI 13.5-20.5) compared to 20.7 (95% CI 19.0-22.6), $t = 1.97$, $p < 0.05$. There

was no association between chicken consumption and any of the measures of severity. Neither were there any significant associations between chicken consumption and the frequency of occurrence of any of the different symptoms: the proportion suffering from muscle pains was larger in the group who had had two or more pieces (22/23 compared with 100/128, $p = 0.08$ Fisher's two-tailed test) but none of the other measurements approached statistical significance at the 5% level.

The age distributions for those who ate one or two pieces of chicken were similar. More men than women ate two pieces of chicken (13/86 compared to 2/35). Adjusting for age and sex did not affect the results of the analyses relating to chicken consumption.

DISCUSSION

In this large, point source outbreak most of the cases were doctors so the descriptions of symptoms are likely to be particularly accurate. The initial questionnaires were sent out only four days after the outbreak so recall of the symptoms and their timing, and of the food consumed, is likely to be good. There is no reason to suspect any particular bias in the responses. All the available measures of severity were analysed so the presentation of results is not biased.

It was striking that all the measures of severity were highly significantly negatively correlated with incubation period, and shorter incubation periods were associated with an increased proportion of patients suffering from most of the different symptoms recorded and with hospitalization. Is this relationship between incubation period and severity due to infecting dose?

The amount of chicken consumed provided another measure of dose which might be thought to be a better proxy for the actual infecting dose. Those who ate two pieces of chicken had on average shorter incubation periods. However, the amount of chicken consumed had no influence on severity of disease. And, as was noted in the previous report, the attack rates were similar for those who ate one or more pieces of chicken (Palmer et al 1990). It is unlikely that the salmonellae would be uniformly distributed among the chicken pieces, so the number of pieces consumed provides only a crude measure of dose. And the difference in dose between one and two pieces is small compared to experimental studies which usually consider

logarithmic increases. The lack of an association with attack rate suggests that the chicken consumption did not accurately reflect dose (McCullough and Eisele 1951acd, Blaser and Newman 1982). In this context the finding of a statistically significant association between the amount of chicken consumed and incubation period is surprising. This contrasts with the Connecticut outbreak of *Salmonella enteritidis* discussed in the Section 2.3 (Mintz et al 1991) where a uniform distribution in the Hollandaise sauce would be expected, and where amounts consumed might have varied several fold.

The incubation period may be a better proxy measure of dose, and has the added advantages of allowing measurement on a continuous rather than a categorical scale and over a larger range of values. However, incubation period will vary with individual susceptibility, and individual susceptibility can also be expected to influence the severity of the disease, as discussed in Section 1.3.

It is not possible to control fully for individual susceptibility. Blaser and Newman (1982) identified eight factors which affect host susceptibility or the proportion of organisms actually reaching the intestine from a given inoculum: age, immune status, debility, prior ingestion of antibiotics, gastric pH, the rate of emptying of gastric contents, the type of vehicle, and the portal of entry. The last two can be ignored as they were identical for all of the patients. Age, prior antibiotic use and factors known to alter gastric pH were controlled for. Variations in host immune status, individual variations in gastric acidity and emptying, and the possible presence of other illnesses remain.

Table 2.4.1. Symptoms reported by ill delegates.

Symptom	% with symptom
Diarrhoea	98.4 (182/185)[1]
Abdominal pain	96.7 (175/181)
Fever	92.0 (161/175)
Headache	82.9 (141/170)
Muscle pains	80.6 (129/160)
Rigors	73.2 (115/157)
Vomiting	62.7 (99/158)
Mucus in faeces	49.5 (54/109)
Paraesthesia	21.1 (23/109)
Blood in faeces	10.4 (10/96)

[1]No. reporting symptom/no. responding to question

Table 2.4.2. The proportion of patients suffering each symptom by incubation period, divided into tertiles.

Symptom or outcome	Incubation period (hours)			χ^2 trend	*p* value
	< 16	≤ 16 to ≤ 22	> 22		
Diarrhoea	100.0% (61/61)	100.0% (65/65)	94.9% (56/59)	4.8	0.03
Abdominal pain	100.0% (61/61)	98.4% (60/61)	91.5% (54/59)	6.6	0.01
Fever	98.2% (56/57)	95.2% (59/62)	82.1% (46/56)	9.9	0.002
Headache	82.7% (43/52)	86.4% (51/59)	79.7% (47/59)	0.2	0.6
Muscle pains	84.3% (43/51)	83.3% (45/54)	74.5% (41/55)	1.6	0.2
Rigors	86.0% (43/50)	75.5% (40/53)	59.3% (32/54)	9.5	0.002
Vomiting	82.1% (46/56)	72.7% (40/55)	27.7% (13/47)	31.0	< 0.001
Mucus in faeces	57.6% (19/33)	50.0% (20/40)	41.7% (15/36)	1.7	0.2
Paraesthesia	16.1% (5/31)	25.6% (10/39)	20.5% (8/39)	0.1	0.7
Blood in faeces	10.3% (3/29)	6.3% (2/32)	14.3% (5/35)	0.3	0.6
Hospitalized	32.2% (19/59)	17.5% (10/57)	5.3% (3/57)	13.9	< 0.001

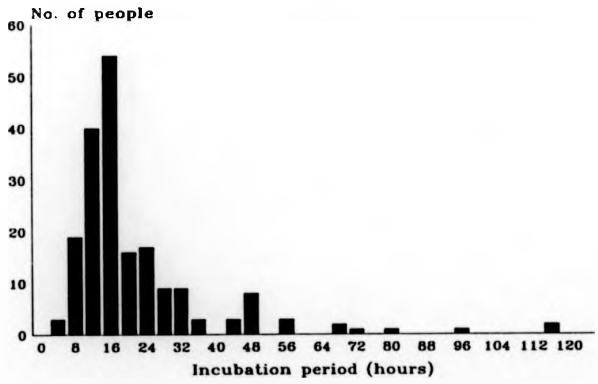


Figure 2.4.1. Incubation periods for the 191 ill delegates in the *S typhimurium* outbreak.

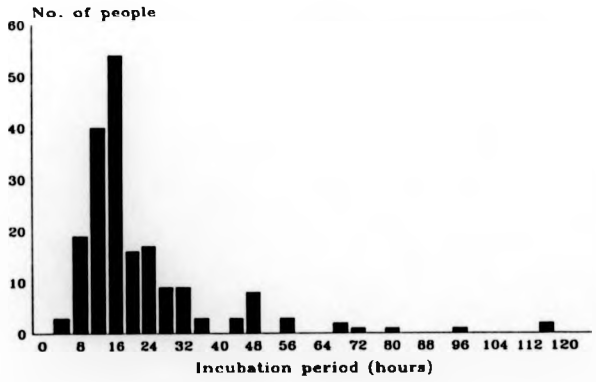


Figure 2.4.1. Incubation periods for the 191 ill delegates in the *S typhimurium* outbreak.

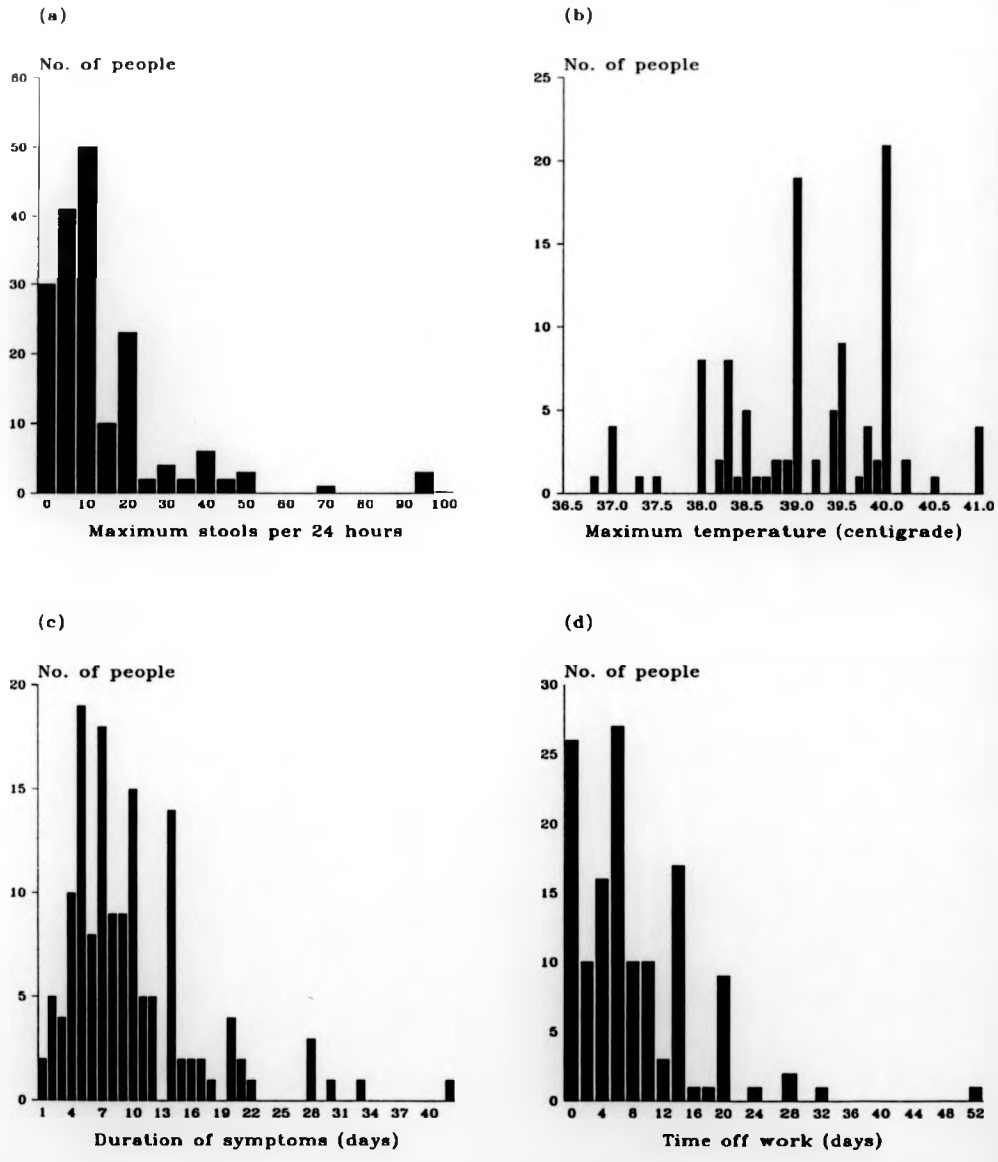


Figure 2.4.2. Distribution of four measures of severity of disease experienced by ill delegates following the *S typhimurium* outbreak. (a) Maximum number of diarrhoea stools per 24 hours (data available for 177 people). (b) Maximum temperature reached (data available for 107 people). (c) Duration of symptoms (data available for 143 people). (d) Time taken off work (data available for 135 people).

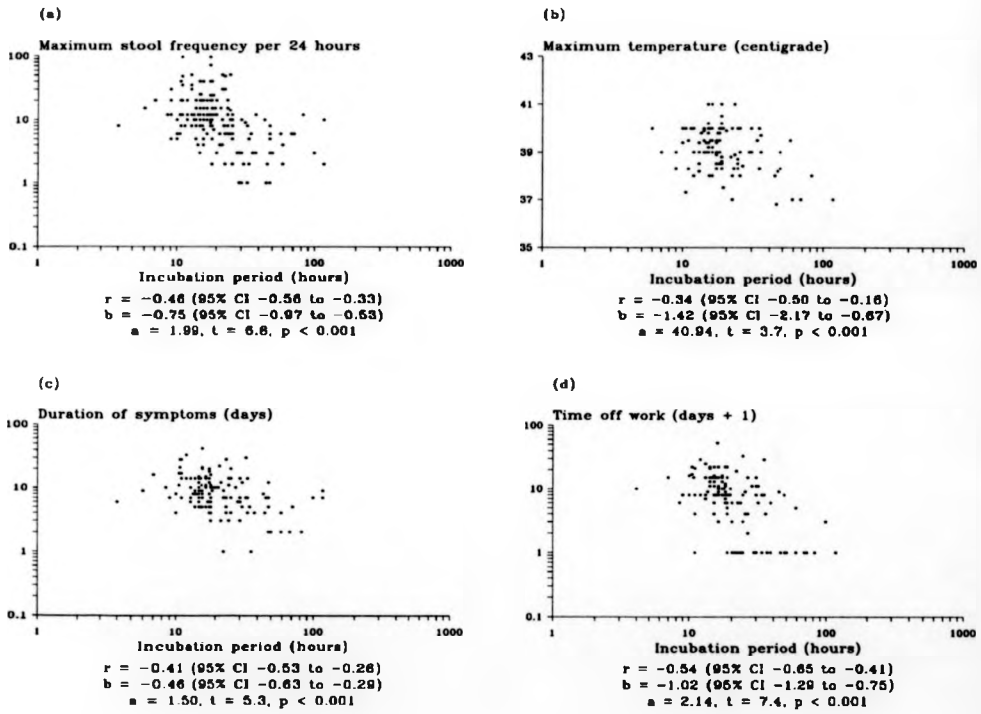


Figure 2.4.3. Relationship between incubation period and four measures of severity, for delegates in the *S typhimurium* outbreak. Linear regression results are shown with each graph.

ANALYSIS OF EXPERIMENTAL TYPHOID DATA

In 1970 Hornick et al summarised their experience of induced typhoid fever in volunteers. They established that larger inocula produced higher attack rates and, on average, shorter incubation periods (Table 2.2.1). They noted that "once illness occurred, the clinical courses were comparable regardless of the dose of the infectious inoculum". No further details were given and it is not clear from the paper how severity was judged nor whether any potential confounders such as age, race or vaccination status were considered when reaching this conclusion. Furthermore, the definition of illness apparently used to make this assessment included an oral temperature of at least 103°F for at least 24 to 36 hours; this would exclude mild cases and leave only a small range of severity available for assessment.

I have returned to the original files, held at the Center for Vaccine Development, Baltimore, and extracted data on all unvaccinated volunteers infected with a known dose of the Quailes strain of *S typhi*.

METHODS

The data now available were collected between 1959 and 1970. The studies were principally designed to test vaccines but I have considered only the volunteers who had not received vaccines or previous inoculations. The methods used in the challenges are described by Hornick et al (1970) and in an unpublished manuscript by WE Woodward. The volunteers were healthy male prisoners from the Maryland House of Correction. The typhoid strain used (the Quailes strain) was isolated in 1958 from the bile of a chronic carrier and was preserved in frozen milk. It is phage type D-1, possessing Vi antigen. It was administered in milk and volunteers were instructed to gargle before swallowing. In the earlier experiments the dose used was varied, but from 1965 a dose of 10^5 (the estimated ID25) was always used. Some of the protocols for individual experiments are now missing and some are very brief. The published account refers to a standard protocol for these experiments, which included starting antibiotics (usually chloramphenicol) when the temperature was at least 103°F for 24 to 36 hours. One important change, however, is that from 1965 volunteers (other than those involved in food-handling) were seen as outpatients unless and until they became ill. The definition of illness resulting in admission was not always given, and the frequency with which the volunteers were seen while outpatients varied between once and twice a day. Inpatients had

their temperatures recorded at 4 hourly intervals during the day, and symptoms and signs were recorded daily on checklists. There was no consistent effort to record the severity of symptoms. For each day a symptom was recorded as 0 (absent), ± (equivocal), or + (present); I classified ± as absent. Although it might have been possible to record the duration of each symptom, it appeared that the charts were filled in more thoroughly at some times than others so little weight could be put on any findings. I have therefore simply recorded whether a symptom or sign was ever present during the illness. Laboratory measures such as culture results, immunological findings and blood counts are present too infrequently in the results to be used in this analysis, or as part of the definition of illness.

Defining illness

I wanted to use a less strict definition of illness than that used by Hornick et al so as to include any cases of mild disease which may have occurred. If the definition of disease requires severe disease, and this is quickly followed by treatment, there is little scope for studying determinants of further variation in severity. On the other hand, if the definition is so mild that any intercurrent illness or a chance false recording is classified as typhoid, some patients will be misclassified. We know from Hornick's results that the attack rate is dose related. At high doses almost all patients fulfil a strict definition of disease, leaving few who could be misclassified. At lower doses fewer patients fulfil the strict criteria leaving many who might genuinely have mild disease or who might be misclassified. Misclassification of non-typhoid as typhoid will tend to bias the results towards a greater proportion of less severe disease at low doses. Misclassification of mild typhoid as not typhoid will bias the results in the other direction (see discussion). I have used different definitions of illness to try and assess the extent of these biases.

Some of the inpatient charts recorded temperatures for several days pre-challenge. Examination of pre-challenge temperatures shows that the measurements tended to be high: peaks over 99°F were not unusual. However, after excluding those where rectal temperatures were used, only one pre-challenge temperature as high as 100°F was recorded, and none of these temperatures were sustained - there was considerable diumal variation with a range of 1-2°F. This normal fluctuation and concern over chance wrong recordings suggested that a sustained fever would be a more suitable measure for defining illness than a single peak.

Another problem in defining illness is the use of out-patient monitoring in the later

experiments. In some protocols patients were only admitted when temperatures over 101°F were recorded and in these same protocols the temperatures were only recorded once a day. In others, temperatures over 100°F were sufficient and in some (those where the patients were seen twice a day) the criteria were not specified. Outpatient temperatures would have been recorded during working hours, whereas the fluctuating temperatures on the 4 hourly charts were often at their highest in the evening, as expected (Petersdorf 1991). To incorporate both the peaks and duration, illness was defined as a temperature of at least 100°F for at least 12 hours continuously and a peak of at least 101°F. I have not included symptoms in the definition because none are specific to typhoid (so would not help to exclude other diseases) and they occurred in different combinations.

Excluding those who started as outpatients leaves very few with the lowest dose resulting in illness (10^5) but is the only way of being sure that all the patients who fulfil a definition of illness with a low threshold are included. For the 10^5 dose, attack rates using the low threshold were compared between inpatients, outpatients seen once a day and outpatients seen twice a day to see if patients with mild disease would be missed if outpatients were included in the analysis.

Using a definition with a higher threshold it is unlikely that patients will be missed if they started as outpatients. It is unclear from Hornick et al's paper if the defining temperature was required to be continuously above 103°F for 24 to 36 hours or if peaks over 103°F over this period were sufficient. The latter is more likely since the sample chart in the paper shows just two brief peaks over 103°F. Applying this criterion to the data gives much lower attack rates than those in the paper. For many of the records the notes record whether the patient was considered ill, though it is unclear if this was noted at the time of the experiment or later. Almost one third of those classified as ill do not fulfil the temperature criteria. Those who were treated with antibiotics were presumably regarded as ill at the time, (with the exception of one patient who was explicitly treated because of prolonged faecal excretion) and the paper notes that some were treated with fevers less than 103°F depending on their other symptoms. Using treatment as the definition of illness should minimize the false positives but will miss cases of mild disease and so will bias the results towards a failure to find a dose effect.

Although volunteers who were vaccinated during the studies were excluded, many of the volunteers had served in the armed forces and so would have received typhoid vaccination at some time in the past. This was recorded and treated as a possible confounder. Other potential

confounders were age, race and year. Year was considered as a confounder because it was strongly correlated with dose and it is possible that changes in protocol other than in the dose, such as in the assessment of patients, could affect the outcome.

RESULTS

Information was available on 278 volunteers who had received inocula ranging from 10^3 to 10^9 organisms. Ages ranged from 20 to 54 years (median 28), 116 were black and 153 white (unrecorded for 9). 102 had been in the military and it was unknown for 70. Among the 88 for whom the date of military service was available, service had finished a mean of 12 years previously (SD 5.9 years, range 0 - 25 years). Among those included as ill, military service finished at least 2 years previously, where recorded. All those challenged after 1965 received 10^5 organisms, compared to only 5 of those becoming ill before 1965.

Attack rate

The attack rate was determined using two different definitions of disease: (1) temperature of at least 100°F for at least 12 hours and a peak of at least 101°F; and (2) treatment given. For the first definition, the few cases where only rectal temperatures were available were excluded, and any patients who, because they were outpatients, had insufficient charts to know whether they fulfilled the criteria, were counted as not ill. (If they were simply excluded the attack rate would be biased. This could be avoided by excluding the whole of that experiment, but that would severely limit the numbers available.) The case where the reason for treatment was stated to be faecal carriage was counted as untreated in the second definition. The attack rates by dose are shown in Table 2.5.1. The relationship between dose and attack rate was independent of age, race and military service. Using both definitions, age group, military service and, to a lesser extent, race were independently associated with attack rates. Attack rates (using definition (1)) were higher in the under thirties (56.6 % (82/145) compared to 31.6% (37/117) in those 30 years or older, RR 1.79, 95% CI 1.32-2.42); in those without military service (54.3% (57/105) compared to 31.3% (30/96) in those with military service, RR 1.74, 95% CI 1.23-2.45); and in Blacks (51.8% (59/114) compared to 39.4% (56/142) in Whites, RR 1.31, 95% CI 1.00-1.72).

For those who received 10^5 organisms the attack rates were compared using definition (1)

between inpatients, outpatients seen twice a day and outpatients seen once a day. The attack rates were similar in the three groups suggesting that few ill patients would be missed by including in the further analysis those who started as outpatients. For the subsequent results only patients meeting the definitions of illness are considered.

Incubation period

The incubation period was taken as the time to the first temperature of at least 100°F, as in the unpublished report. (A few patients had spikes of temperature over 100°F on the first or second day after challenge as shown in Hornick et al 1970, figure 1: these were ignored.) The incubation period was log normally distributed and was inversely related to dose (Table 2.5.2) and the results were similar using the two different definitions of illness. The correlation persisted when outpatients were excluded and was similar if the first temperature of 101°F was used rather than 100°F. There were no associations between age, race or previous military service and the incubation period, and adding these potential confounders to the regression equation produced little change in the regression coefficients.

Severity of illness

The severity of illness was assessed by several measures. The most objective measures related to the temperature: the peak temperature reached and the duration of temperatures over 103°F. Symptoms and signs were considered individually and as the total number occurring in each individual (the symptom score). The peak temperature and symptom score were approximately normally distributed. The duration of temperatures over 103°F was skewed to the right.

Illness defined using definition (1): 120 cases

The peak temperature was positively correlated with log dose (Table 2.5.3): r = 0.22 (95% CI 0.04-0.39), b = 0.13, (0.027-0.24). When outpatients were excluded (leaving only 12 patients in the 10^5 dose group, 49 in all) the correlation was stronger: r = 0.44 (0.18 to 0.64), b = 0.28 (0.16-0.45). The regression coefficients were hardly altered by adjusting for the age, race or military service, and became stronger after adjusting for year.

The time period when the temperature was continuously above 103°F was assessed from the temperature charts and so is presented in terms of the number of 4 hour periods. The duration was longer in the highest dose group (Table 2.5.3) but the correlation (with log number of 4

hour periods + 1) did not reach significance at the 5% level (r = 0.13, -0.05 to 0.31). After excluding outpatients the trend was stronger: r= 0.31 (0.03-0.55). There was no evidence of confounding.

The symptom score increased with dose (Table 2.5.3): r = 0.27 (0.09-0.43), b = 0.39 (0.14-0.64). There was no evidence of confounding by age, race or military service, but the association was lost after adjusting for year (b = 0.27 (-0.12-0.65) and when outpatients were excluded: r = 0.13, (-0.16 to 0.40), b = 0.17, (-0.20 to 0.55).

The 18 individual symptoms are shown in Table 2.5.4. Only lymphadenopathy showed a significant increase with dose, but the proportion was highest in the middle dose group and the association was lost after adjusting for year in a logistic regression analysis. The trend for abdominal pain also approached significance but again was decreased by adjusting for year. The results were little altered by stratifying by age group, race or military service. When outpatients were excluded the results were similar, with a non significant trend for lymphadenopathy, a significant trend for anorexia and no other results approaching significance at the 5% level. Excluding outpatients leaves few patients from the later years, and adjusting for year had little effect.

Because the symptoms sometimes started late, occasionally some days after a brief initial rise in temperature, relapses were defined as a recurrence of fever and symptoms occurring after completing a course of treatment. There was no correlation between dose and relapse rate in the full data set (Table 2.5.4) or after excluding the outpatients. Treatment was given more commonly in the higher dose groups, and this trend was more marked after excluding the outpatients and after adjusting for year. There was no other evidence of confounding in these relationships.

There was no evidence of associations between age, race or previous military service and any of the major outcomes: peak temperature, duration of temperature over 103°F or symptom score. Time from military service was only known for 28 cases (under definition (1)). There were no correlations between time from service and any of the major outcomes.

The year of the experiment was weakly positively associated with peak temperature, after adjusting for dose. There was no correlation with duration of high temperatures. The mean symptom scores were compared by year and by protocol among those who became ill after 10^5

organisms. There were no significant differences between the groups, and no evidence of a trend towards increased or decreased recording over time. Among all those who were ill, an inverse correlation between year and symptom score was lost after adjusting for dose. The signs (hepatomegaly, splenomegaly, jaundice, lymphadenopathy and rose spots) were separated from the symptoms and considered separately as a more sensitive measure of how thoroughly the patients were assessed over time. The proportion of patients with at least one sign decreased with the year of experiment, even after allowing for dose.

Illness defined using definition (2): 115 cases

Six patients with only rectal temperatures recorded were included using this definition; all received 10^9 organisms. The peak temperature for these patients was estimated as the recorded temperature minus 0.7°F, based on the estimated difference between oral and rectal temperatures given by Petersdorf (1991). They were excluded from the analysis of duration of fever.

There was no correlation between log dose and peak temperature, including or excluding those with rectal temperatures, with or without adjusting for the confounders: r = 0.08 (-0.10 to 0.26). There was no correlation between log dose and log duration of fever over 103°F: r = 0.06 (-0.13 to 0.25). Again there was no evidence of confounding.

The symptom score increased with log dose: r = 0.25 (0.07 to 0.42), b = 0.33 (0.10 to 0.56). There was no evidence of confounding by age, race or military service, but the association was lost after adjusting for year. Among the individual symptoms a significant trend was only found for lymphadenopathy and this was lost after adjusting for year; no other trends with symptoms approached significance. Relapse rates were similar in all dose groups. Treatment was, by definition, given to all patients. Drugs other than chloramphenicol were grouped together and drug type was considered as a possible confounder: this had no influence on the results.

Exploration of possible bias introduced by the definition of illness

Taking the three main measures of severity - peak temperature, duration of temperatures over 103°F, and symptom score - bias introduced by the illness definition was explored further by comparing the results obtained using definition (1) with those obtained by using two more definitions based on temperature: a temperature of at least 101°F for 12 hours continuously (definition (3)); and a temperature peaking to at least 103°F over 36 hours (to approximate to

the definition in the paper (Hornick et al 1970) - definition (4)). The results are shown in Table 2.5.5.

DISCUSSION

The relationships reported by Hornick et al (1970) between dose and attack rate and incubation period were confirmed in this analysis and persisted after adjusting for potential confounders. The published report found no evidence of an association between dose and severity. Is this borne out by this more detailed analysis?

Some of the protocols are missing from the data, and some are very brief. I think all the patients included received the Quailes strain, although it is not always explicit in the notes. The criteria on which the assessments of the presence of symptoms and signs were based are not recorded. It is possible that they changed over the course of the experiments - certainly the charts were not always filled in by the same person. Although the total number of symptoms showed little systematic change over time or with protocol, after allowing for dose, the recording of signs decreased with year independently of changes in dose. Year has been treated as a confounder, but because it is so closely correlated with dose it is difficult to disentangle the two effects.

The bias introduced by the illness definition is potentially serious. The action of this bias can be illustrated in the following theoretical examples. Suppose there are 50 patients in a low dose and 50 patients in a high dose group and they become ill as shown:

	Low dose	High dose
Not ill	40	20
Ill	10	30
(Mild illness)	(2)	(6)

In this situation there is no dose-severity relationship and among those who are ill 20% have mild symptoms. Now suppose 10% of those who are not truly ill with the disease in question have mild symptoms and are classified as ill. This gives the situation:

	Low dose	High dose
Not ill	36	18
Ill	14	32
(Mild illness)	(6)	(8)

The illness is now apparently severe in 24/32 (75%) of those who received the high dose and 8/14 (57%) of those who received the low dose: a spurious dose-severity effect. The illness definition used must have a high specificity to minimise this bias.

Now suppose that there is a dose severity relationship so that the "true" situation is as shown:

	Low dose	High dose
Not ill	40	20
Ill	10	30
(Mild illness)	(6)	(6)

In this scenario severe disease occurs in 80% after the high dose and 40% after the low dose, a relative risk of 2. If 50% of those with mild disease are misclassified as not ill we get:

	Low dose	High dose
Not ill	43	23
Ill	7	27
(Mild illness)	(3)	(3)

Severe disease is now seen in 89% after the high dose and 57% after the low dose, a relative risk of 1.6. The relative risk is biased towards no effect.

Table 2.5.5 shows that the biases are acting in the direction predicted: as the illness definition becomes stricter the correlation coefficients move towards zero. Only the correlation with number of symptoms persists in all definitions but this could be due to changes in assessing patients over the years. There was little evidence of correlations between dose and individual symptoms, apart from lymphadenopathy (which could again be due to year). In particular, symptoms and signs which might in themselves indicate severe disease - photophobia, hepatomegaly and splenomegaly - were no more frequent after higher doses.

The correlation with peak temperature was less robust under different illness definitions. The results using the 1st and 3rd definitions suggest a correlation but there was no evidence of a correlation among those who were treated. The duration of temperatures over 103°F was only weakly correlated with dose, but this is perhaps not surprising since, following the protocol, patients were treated after a high fever of a certain duration. Relapse rates were similar at all doses.

The changes in results seen between the definitions depend on the classification of only a few patients. 12 patients included in definition (1) were not treated, and (excluding the patients with rectal temperatures) 1 patient was included in definition (2) who was not in definition (1). Of the 12, 9 had peak temperatures of at least 102°F. The three others had symptom scores of 2, 5 and 6. The patient with the lowest score had a temperature over 100°F for over 24 hours. It seems likely that the majority if not all of these patients had typhoid. In fact some typhoid cases may be missed even by definition (1) as suggested by the one treated case who did not fulfil the criteria. Nine additional patients had peak temperatures of at least 101°F but did not meet the criteria for definition (1) and some or all of these may have had typhoid. If all patients with peak temperatures of at least 101°F are regarded as ill the regression coefficients obtained between dose and the three major symptoms are very similar to those under definition (1).

In conclusion, the first definition seems to provide a reasonable compromise between sensitivity and specificity. The sensitivity of the other definitions is probably too low, but where trends persist under these definitions it gives weight to the findings. In these studies with the Quailes strain of *S typhi*, over the very large dose range used, there is some evidence of an effect of dose on peak temperature and number of symptoms, though the latter could be due to confounding by year. The correlations found were weak, and although they reached conventional levels of statistical significance this should be interpreted cautiously in view of the large number of comparisons carried out. There was little evidence of correlations between dose and duration of high fever, and dose did not predict the occurrence of individual signs or symptoms indicative of severe disease, nor of relapse.

This analysis has illustrated the problems of defining disease and thereby the difficulties of separating the known influence of dose on attack rate from the unknown influence of dose on severity of disease.

Table 2.5.1. Attack rate by challenge dose. For illness definitions see text. For the second definition 4 patients who received 10^6 organisms (and only had rectal temperatures recorded) are included in the 10^5 group. None of these 4 became ill.

Dose	Ill by 1st definition		Ill by 2nd definition	
10^3	0/13	(0 %)	0/13	(0 %)
10^5	83/200	(41.5%)	72/204	(35.3%)
10^7	13/27	(48.1%)	13/27	(48.1%)
10^8 - 10^9	24/25	(96.0%)	30/34	(88.2%)
χ^2 trend	33 ($p < 0.001$)		41 ($p < 0.001$)	

Table 2.5.2. Incubation period by dose. Illness defined by definition (1).

Dose	N	Geometric mean incubation period in days (95% CI)
10^5	82	9.35 (8.38 - 10.44)
10^7	13	7.40 (4.89 - 11.20)
10^8 - 10^9	24	4.69 (4.07 - 5.40)

Table 2.5.3. Relationships between dose and peak fever, duration of temperature above 103°F, and symptom score. Illness defined by definition (1).

		Dose		
		10^5	10^7	10^8 - 10^9
Peak temperature reached (°F)	N	83	13	24
	Mean	103.81	103.62	104.40
	95% CI	103.60-104.02	103.13-104.10	104.07-104.73
Time above 103°F. (No. of 4 hour periods + 1)	N	80	13	23
	Geometric mean	3.2	2.6	4.8
	95% CI	2.5-4.0	1.4-5.0	3.4-6.7
Symptom score	N	83	13	24
	Mean	7.7	8.9	9.2
	95% CI	7.2-8.2	7.8-10.0	8.3-10.0

Table 2.5.4. The percentage of patients with each outcome, by dose. Illness defined by definition (1)

Outcome	Dose			χ^2 trend	*p*
	10^5	10^7	10^8 - 10^9		
	N = 83	N = 13	N = 24		
Anorexia	90.4	92.3	100.0	1.7	0.2
Nausea	51.8	46.2	66.7		
Vomiting	31.3	38.5	41.7		
Abdominal pain	74.7	92.3	91.7	3.6	0.06
Diarrhoea	27.7	38.5	45.8	2.6	0.1
Constipation	56.6	38.5	70.8		
Hepatomegaly	14.5	23.1	12.5		
Splenomegaly	7.2	0.0	8.3		
Jaundice	0.0	7.7	0.0		
Cough	59.0	53.8	54.2		
Sore throat	38.6	61.5	54.2	2.2	0.1
Headache	97.6	100.0	95.8		
Sweats	78.3	84.6	79.2		
Malaise	78.3	76.9	91.7	1.4	0.2
Rigors	6.0	7.7	4.2		
Photophobia	34.9	38.5	33.3		
Rose spots	3.6	7.7	8.3		
Lymphadenopathy	19.3	84.6	58.3	17.6	<0.001
Treatment	86.7	92.3	100.0	3.0	0.08
Relapse	19.3	23.1	26.1		

Table 2.5.5. Correlations between the three main outcomes and log dose under three different definitions of illness.

Outcome		Definition (1) ≥ 100°F for 12 hrs + peak ≥ 101°F (N = 120)	Definition (3) ≥ 101°F for 12 hrs (N = 112)	Definition (4) Spiking over 103°F for 36 hrs. (N = 78)
Peak temperature	r (95% CI)	0.22 (0.04 - 0.39)	0.18 (0.00 - 0.35)	0.17 (-0.05 - 0.38)
	b (95% CI)	0.13 (0.03 - 0.24)	0.09 (0.00 - 0.19)	0.07 (-0.02 - 0.16)
Log time over 103°F*	r (95% CI)	0.13 (-0.05 - 0.31)	0.09 (-0.09 - 0.28)	0.03 (-0.20 - 0.25)
	b (95% CI)	0.04 (-0.01 - 0.09)	0.03 (-0.03 - 0.08)	0.01 (-0.05 - 0.06)
Symptom score	r (95% CI)	0.27 (0.09 - 0.43)	0.24 (0.06 - 0.41)	0.22 (0.00 - 0.42)
	b (95% CI)	0.39 (0.14 - 0.64)	0.33 (0.08 - 0.57)	0.26 (0.00 - 0.52)
	b' (95% CI)†	0.27 (-0.12 - 0.65)	0.09 (-0.29 - 0.46)	-0.12 (-0.56 - 0.32)

* ie log (no. of 4 hour periods when temperature ≥ 103°F continuously + 1)
† partial regression coefficient, adjusted for year of experiment

CONCLUSIONS

For typhoid the evidence from the volunteer studies suggests that there might be a correlation between dose and severity. No evidence for a relationship was found in the comparison of published epidemics. There are several explanations for this difference. The deficiencies of the published typhoid epidemic data and the inherent biases have been discussed at length. In the well controlled conditions of studies in selected volunteers using a large dose range of a single strain of typhoid it was only possible to demonstrate a weak effect. The failure to detect such a weak effect in the published studies with their more varied patients and probably smaller dose ranges is not surprising. We saw how, in the volunteer data, the correlation with peak temperature was lost when a strict definition of disease was used. In natural outbreaks mild cases are very likely to be missed.

The outcomes considered in the two studies were very different. In the volunteer studies two of the three major outcomes considered related to fever. Illness was defined on the basis of fever (even when treatment was the basis of the definition, treatment was given in response to fever) and attack rates were dose-related. High fevers are part of the same pathogenic process that determined "illness" and might also be expected to correlate with dose. Mortality in typhoid can arise in a number of different ways depending on different pathogenic processes, some remote from those which lead to diagnosis. Variation in host factors might be expected to be relatively more important. These points are explored further in the final discussion.

For the food-poisoning salmonellae the evidence as a whole - from individual epidemics and from the comparison of hospitalization rates - suggests that there is a dose-severity relationship at least for *S enteritidis*, *S typhimurium*, *S infantis*, *S newport* and *S thompson*.

This contrast between a dose-severity effect for food-poisoning salmonellae and a weak effect (if any) for typhoid, is reflected in the differing response to challenge of subjects who are partially immune. In volunteer experiments with typhoid, vaccines gave protection against low but not high challenge doses, but once clinical disease occurred the severity of the disease and the number of relapses were not altered by vaccination (Hornick et al 1970, Hornick & Woodward 1966). This is consistent with dose influencing only the proportion of people becoming ill and not the severity of the infection. However, for the food-poisoning *Salmonellae*, when subjects who had become ill were rechallenged, if they became ill again the severity of the illness was usually less than that of the initial illness, despite higher challenge

doses being used (McCullough & Eisele 1951b). This change in severity with immunity is consistent with a dose-severity relationship.

SECTION 3

MALARIA

INTRODUCTION

Whether inoculum size influences severity of infection in malaria has been debated for many years. Malaria can vary in severity from asymptomatic parasitaemia to a fatal multisystem disease. Known variations in virulence between malaria species and strains and known variations between hosts in resistance due to immunity, nutritional status, age and red cell disorders are insufficient to explain the range in outcome (Warrell et al 1990, Hayes et al 1992).

In theoretical terms, infecting dose might be expected to influence the severity of disease in malaria. During the blood stage of infection with malaria, the increase in parasite numbers is initially logarithmic (MaeGraith 1948). The rate of rise will be the same whatever the initial infecting dose, but since the infecting dose determines the starting point, the time taken to reach a given level of parasitaemia will be dose-dependent (Hamilton Fairley 1947). Following a low dose infection, the immune response, which is time-dependent, should start to take effect while the parasite load is still low, and might therefore be more effective in limiting the severity of disease (Greenwood et al 1991, Marsh 1992).

The question has assumed new importance with the increasing use of insecticide-impregnated bednets (Bermejo & Veeken 1992). It has been suggested that the success of impregnated bednets in preventing clinical malaria but not parasitaemia in some studies could be due to a reduction in the sporozoite inoculum (Snow et al 1988, Greenwood et al 1991). Induced malaria can provide evidence of whether a dose-severity relationship exists, since the dose can be estimated. This section starts with a review of the published evidence available for induced malaria in man and animals in which it is possible to compare dose and outcome. This is followed by an analysis of previously unanalysed data extracted from the records of patients given therapeutic malaria. Finally the results are discussed in relation to the pathogenesis of malaria and the findings from bednet studies.

LITERATURE REVIEW

INDUCED HUMAN MALARIA

Malaria can be induced in man and other animals by one of three methods: by the bites of infected mosquitos, by injecting sporozoites extracted from mosquitos, or by injecting infected blood from a subject with malaria. All of these methods have been used at different times and all allow some degree of quantification of the dose received.

Manson in 1900 fed infected mosquitos on his son to prove to a sceptical British public that mosquitos do indeed carry malaria (Manson 1900). Human volunteers have been used at other times, notably by Hamilton Fairley in Cairns, Australia (Hamilton Fairley 1947), and by Coatney in the United States (Coatney et al 1950a,b). However the bulk of the experience with induced human malaria comes from the use of malaria therapeutically in the treatment of neurosyphilis. The benefit to the patients was thought to come from the fever or from some other effect of the parasite, so the malaria was allowed to proceed often for ten or more paroxysms without treatment, unless the patient became too unwell (James 1931). This was obviously not without its dangers; one report gives a fatality rate of 10% for therapy with *Plasmodium vivax* in England (Whelen & Shute 1943). Other series found rates around 5%, but they could be as high as 15-20% in the early years (Fong 1937).

The benefit of malaria therapy to neurosyphilis patients is hard to assess as no formal trials were done. The outlook without the therapy was variable but often bleak. One study estimated that 3-12% of untreated paretics underwent spontaneous remissions but 42% were dead within a year of admission (Chernin 1984). A recent review estimates that with malaria therapy 10-60% of patients achieved remission, 10-50% showed partial improvement, and 15-40% failed to improve (Chernin 1984).

Malaria therapy was used from the early 1920s until after the second world war, when the introduction of penicillin gradually made it obsolete (Covell 1956). Malariologists at the time realised the potential importance of this therapy for furthering malaria research. Their interests centred on the life-cycle of the parasite and in particular the mechanism of recurrences, and on assessing the efficacy of different treatments. Among the large number of articles produced there is some information relating to the effects of dose. In some cases dose effects are referred

to specifically, in others I have derived the information from the data presented. Studies were identified using the *Tropical Diseases Bulletin* from 1925 onwards and following up references. All identified studies containing sufficient information have been included.

Many of the problems in interpreting the results recur in different studies so they are summarised below.

1) Problems of estimating dose in mosquito-induced infections

The most obvious way of assessing dose in infections caused by mosquitos is to count the number of bites or of mosquitos employed. This however assumes that all the mosquitos are infected and transmit the same number of sporozoites, neither of which is likely to be the case.

The problems of infecting mosquitos from donors known to have gametocytes is discussed at length in the early literature (James 1926, James 1931). Shute (1937) noted that in one experiment when 30 mosquitos fed on the same patient who was known to have gametocytes in the blood, subsequent dissection showed that three of the mosquitos remained negative and that the range in number of oocysts in the others was from 1 to 800. In another study (Shute 1945) fifty mosquitos were fed on the same gametocyte carrier; 44 became positive, and the sporozoite counts in the salivary glands ranged from 11,450 to 219,450.

In MF Boyd's studies in the USA, the mosquitos were dissected after use and the numbers given refer to infected mosquitos. In some of Boyd's studies some mosquitos from each batch were dissected without having fed on patients and the number of oocysts in the stomach counted. This allowed classification of the batches into grades, but assumed that all the mosquitos in a batch were infected to the same degree (Boyd & Stratman-Thomas 1933b, Boyd & Kitchen 1937b,c, Boyd 1940b).

Two American groups measured the degree of infection in the salivary glands of the mosquitos used in experimental infections in prisoner volunteers (Whorton et al 1947a, Coatney et al 1950a,b). Both used a scoring system for sporozoites. The salivary glands of the mosquitos used were afterwards scored subjectively for level of infection from 1+ to 4 or 6+, and the dose received by the volunteer was taken as the sum of pluses of the mosquitos involved. In summing the pluses from the mosquitos used, it was implicitly assumed that in terms of sporozoite numbers two mosquitos scoring 2+ each were equivalent to one mosquito scoring

4+. No actual sporozoite numbers were given, so the validity of this assumption cannot be assessed, even in terms of gland loads.

Neither the count in the glands nor the oocyst number are necessarily closely related to the number of sporozoites inoculated. Recent experiments inducing mosquito salivation of *P falciparum*-infected *Anopheles stephensi in vitro* have suggested that the number of sporozoites ejected is considerably smaller than the number in the glands (Rosenberg 1990). Median counts of 15 sporozoites in the saliva were obtained from mosquitos with median gland counts of around 8000. The number ejected was highly variable, ranging from zero to nearly one thousand, however there was a positive correlation between the number ejected and the number in the glands.

Subsequent studies have given similar results. Beier et al found similarly low ejects (geometric mean ejects between four and six sporozoites of *P falciparum*) for both laboratory and naturally infected mosquitos using a capillary tube technique to gather saliva (Beier 1991 a,b). In different experiments, between 15 and 75% of infected mosquitos failed to transmit any sporozoites. Mosquitos with larger salivary gland loads were more likely to transmit sporozoites, and for *An gambiae*, but not *An stephensi*, there was some correlation between the gland load and the number of sporozoites transmitted. Using a similar technique with *P berghei*-infected *An stephensi*, Li et al (1992) found that the mean number of sporozoites ejected (32-36 sporozoites) was not influenced by previous probing of the mosquitos on rats.

Ponnundurai et al (1991) fed *P falciparum*-infected *An stephensi* on fresh mouse skin placed over blood to simulate normal feeding more closely, and counted the sporozoites in the skin and in the blood. In these experiments 9 of 34 infected mosquitos failed to transmit any sporozoites, and the median number ejected was 8 (range 0-522). The sporozoite gland count did not correlate with the number ejected.

Two further, indirect studies have come to similar conclusions about inoculum size. Vanderberg (1977) calculated the number of sporozoites per *An stephensi* mosquito from the number of extra-erythrocytic forms of *P berghei* in the livers of mice and rats at 42 hours after the inoculation. The mean numbers of extra-erythrocytic forms per mosquito were 24 in mice and 97 in rats. There was a correlation between the mean intensity of salivary gland infections in the mosquitos and the mean number of extra-erythrocytic forms per mosquito in different experiments. Davis et al (1989) back-calculated from the level of *P falciparum* parasitaemia in

four volunteers to find an average inoculum of 120 sporozoites from five mosquito bites, or 24 per mosquito.

Overall, it appears that the inocula are very small, and that there is a weak association between inoculum size and salivary gland load. In Beier's experiments over 80% of the mosquitos transmitted fewer than 15 sporozoites, with only a few transmitting over 100. Given the variability in sporozoite inoculum between mosquitos, the variation in number of mosquitos used to induce malaria is small, and deductions on relative dose very crude. No studies relate oocyst number to eject size.

Another problem is the decrease in sporozoite numbers over time. In a batch of mosquitos all infected at the same time, the proportion with sporozoites in their glands gradually decreases (James & Shute 1926). Using *P gallinaceum* Porter et al (1954) showed that both the number of sporozoites and their infectivity decreased over time.

Boyd's group refrigerated mosquitos while they were waiting to be used, after their infection had matured (Boyd & Stratman-Thomas 1934, Boyd et al 1936). They found that the proportion of successful infections fell with increased storage time above 20 days for *P vivax* and above 10 days for *P falciparum* (Boyd & Kitchen 1937b).

2) Problems of estimating dose with direct sporozoite inoculation

In principle, the most accurate way of knowing the number of sporozoites inoculated is to inject the sporozoites directly. Early in the practice of malaria therapy James et al (1927) described a technique for extracting sporozoites from mosquito salivary glands, and Shute (1937) gave a fuller description of the technique and added a method for determining the number of sporozoites injected. Shute did not mention whether any checks were made on the accuracy of the method, and problems were experienced by another author when he attempted to repeat it (Roy 1938). In addition, handling the sporozoites may have affected their viability, so the numbers recorded may be a poor approximation to the number of viable sporozoites.

3) Problems of estimating dose in trophozoite-induced infections

The commonest method for inducing malaria for malaria therapy was by blood inoculation from one patient to another. Often strains of malaria were maintained for long periods by

repeated subinoculations. As a known amount of blood was injected and parasite counts could be made on it, it was relatively easy to calculate the size of the inoculum. Of course, this does not provide direct information on mosquito-induced infections but it is trophozoites that are transferred in vertical transmission of malaria, and in malaria following blood transfusions.

The blood groups of donors and recipients are important: it has been shown clearly that the incubation period was longer in patients who received incompatible blood than in those who received the same amount of compatible blood (Polayes & Derby 1934, Myatt et al 1954). The early literature was summarised by Myatt et al (1954).

Horn (1929) studied the microscopic appearances of malaria parasites in a donor's blood when exposed to incompatible serum. Several morphological changes were seen, including shrinking and breaking up of the parasites. This suggests that parasites were killed when an incompatible blood inoculation was given, so the dose received by the patient was effectively smaller.

Unfortunately, few of the studies which relate dose of trophozoites to outcome take blood group into consideration, and in the three studies that do consider blood group as well, the only outcome recorded is the incubation period (v. Assendelft 1934, Beltran & Sandoval 1946, Whorton et al 1947a). Studies which only report blood volume and neither blood group nor parasite number provide too crude an estimate of dose and are not included in this review.

4) Problems in interpretation arising from the pooling of results

In many of the papers, and particularly those by MF Boyd, the results quoted relate to more than one strain of malaria, and pool the results from primary and subsequent inoculations, and from both black and white patients. It is not always explicit in the papers that this has been done, and it only becomes apparent by comparison with other papers referring to essentially the same group of patients (compare Boyd & Kitchen 1937a with Boyd & Kitchen 1937b,e and Boyd 1941). In reporting the results I have tried to clarify this where possible.

5) Problems in interpretation due to the use of neurosyphilis patients

Whether induced malaria in neurosyphilis patients differed in any way from natural malaria was debated at the time (Winckel 1941, Boyd 1944a). Apart from the observed lack of latent infections following trophozoite inoculation and a feeling that trophozoite-induced infections

were easier to treat, no major differences were noted. Not all of the patients given "therapeutic" malaria had neurosyphilis, and the diagnoses were sometimes unspecified. I have specified whether patients or volunteers were used.

The relationship between dose and prepatent and incubation periods

P vivax

Mosquito-induced infections

Although many studies reported prepatent or incubation periods as outcomes in relation to the number of mosquitos used or the degree of infection in the mosquitos, few restricted their results to primary inoculations and single strains. Different strains may have different prepatent and incubation periods, and partially immune patients tend to have longer incubation periods (James 1931). All the larger studies which used non-immune patients and single strains showed inverse correlations between the estimated dose and the prepatent or incubation period, although with considerable variation between individuals.

For 746 patients bitten by between 1 and 30 mosquitos James (1931) found the number of bites to be negatively correlated with the number of days of incubation (correlation coefficient given as -0.157 ± 0.025: the 0.025 is probably the standard error).

Coatney et al (1950b), using prisoner volunteers, found longer prepatent and incubation periods (time to first temperature of 101°F) in those infected by the bites of one mosquito than among those bitten by ten (Table 3.2.1).

In a recent Chinese study, all the volunteers developed malaria, but whereas the 6 volunteers bitten by 10 mosquitos developed malaria within the usual incubation period, half of 24 volunteers bitten by one mosquito developed malaria only after a long latent period. Among those with non-latent infections, the incubation periods were longer in those bitten by only one mosquito (Li et al 1989).

Whorton et al (1947a) used a scoring system based on salivary gland sporozoite loads in experimental infections of prisoner volunteers with the Chesson strain of vivax malaria; all the volunteers were white, and none had a previous history of malaria. Higher scores were associated with shorter prepatent periods.

Coatney et al (1950a) used the St Elizabeth strain and a similar scoring system in 50 prisoner volunteers. The paper mentions that each mosquito was usually allowed to feed on three subjects in turn before being dissected, which may affect the results. The prepatent period was found to be inversely related to inoculum size. An additional three volunteers in the lowest dose group developed infections with latent periods of over 9 months.

Boyd and Kitchen (1938) presented data on patients bitten consecutively by the same group of mosquitos with a 3-4 day interval between each biting session. The first and third patients bitten by each group of mosquitos were thought to be fully susceptible to vivax malaria. (This arrangement of biting was designed to test the immune status of the middle patients.) Between the first and third sets of bites some of the mosquitos from the group died, so the patients bitten third were usually bitten by fewer mosquitos than those bitten first. This, and any sporozoite depletion caused by time and the consecutive bites, should have ensured that the patients bitten on the third application received fewer viable sporozoites than those bitten on the first application. Although this study was probably not restricted to a single strain, the proportion of patients receiving each strain will have been similar in each group. The 47 patients bitten on the first application had, on average, shorter incubation periods than the 43 bitten on the third application.

Direct sporozoite inoculation

Few papers report the results of direct sporozoite inoculations. Mayne (1933) gave only crude assessments of the number of sporozoites, and there was a large degree of variation in the time for which the sporozoites were maintained *in vitro* before being used: no clear relationships with prepatent or incubation period were seen among the 10 patients studied. Two surprisingly recent papers report the results of sporozoite injections in psychiatric patients in Romania. Using a North Korean strain of *P vivax*, low doses were more likely to give rise to latent infections (Shute et al 1976). With the Chesson strain no latent infections occurred among the 11 patients inoculated. Both the prepatent and the incubation periods were inversely related to inoculum size in the range 10-10,000 sporozoites (Ungureanu et al 1976).

One other paper gives some results for sporozoite inoculation with *P vivax*, using an indirect method. Hamilton Fairley (1947) compared infections in volunteers bitten by 11-21 mosquitos to a second group of volunteers infected by subinoculation of blood from volunteers in the first group during the first hour after the mosquito bites. All of the six volunteers in the first group and 3 of the six in the second group developed malaria. The recipient in each pair presumably

received a smaller dose of sporozoites than the donor. For the three pairs where the recipient developed malaria the prepatent periods in the donors were 12, 10 and 12 days, and in the recipients 17, 13 and 36 days.

Trophozoite-induced infections

For trophozoite-induced malaria the main problem in interpreting results is that few studies take account of whether compatible or incompatible blood was used, as discussed above. Of the studies which use trophozoite number, most found an inverse relationship with incubation period (v. Assendelft 1934, Whorton et al 1947a, Boyd 1940a, Kaplan et al 1946a, Hoch et al 1940). In one small study no relationship was found (Grieg & Neill 1939), and Beltran & Sandoval (1946) found a correlation among those who received compatible blood but not incompatible blood.

In one of the larger studies it is clear that a single strain was used (Whorton et al 1947a). 121 psychotic patients in Illinois were given the Chesson strain of *P vivax* intravenously. The incubation period (time to the first occurrence of a rectal temperature over 100°F) was shorter in those who received compatible blood, and was inversely related to dose within the compatible and incompatible blood groups (Table 3.2.2).

P falciparum

Mosquito-induced infections

Four papers by Boyd and Kitchen gave results of their experience of induced falciparum malaria using mosquitos (Boyd & Kitchen 1937a,b,e, Boyd 1941). The results in the 1941 review article repeat those given in earlier papers and the results in the other papers seem to be based on largely the same group of patients. Most of the patients were Black, and most of the results refer to infections with five different strains. The first paper (1937a) states that both first inoculations and second inoculations were included; in the other papers this issue is not mentioned.

Successful take rates were around 60% and did not vary with the number of mosquitos employed (in the range 1-15) (Boyd & Kitchen 1937a,b). For the Coker strain and overall the prepatent period did not vary with the number of mosquitos used, but for the Long strain, larger numbers tended to give shorter prepatent periods (Table 3.2.3).

Incubation periods are only given in one of the papers (Boyd & Kitchen 1937a) and are not divided by strain. The average time to the first elevation of temperature above 100°F showed an increase with increasing numbers of mosquitos, but the confidence intervals of the geometric means were wide and showed considerable overlap.

Jeffrey et al (1959) divided their results by strain and found shorter prepatent and incubation periods with increasing numbers of mosquitos in neurosyphilis patients (Table 3.2.4).

Direct sporozoite inoculation

Covell et al (1949) reported the results of 30 intravenous inoculations with known sporozoite doses in patients receiving malaria therapy. They used a West African strain of *P falciparum* and each patient received the sporozoites from the glands of two mosquitos. All of the patients had received previous malaria therapy but that had taken place at least nine years earlier. For some, falciparum malaria had been used, but only European strains. Most of the patients had prepatent and incubation periods of 7 days. There was an inverse association between dose and incubation and prepatent periods (Table 3.2.5).

Trophozoite-induced infection

I have not found any papers which provide information about inoculum size (in terms of blood volume and parasite count or blood compatibility) in trophozoite-induced falciparum malaria.

P malariae

It proved difficult to transmit *P malariae* to mosquitos, at least to those commonly employed, so experience with mosquito-induced infections is very limited (Shute 1951). No paper gives the results and doses for more than a few patients (eg Ciuca 1964), so no conclusions can be drawn.

Only three papers give quantitative results for trophozoite infections with *P malariae*. Ciuca et al (1943) describe experience with 293 patients. They found that the parasite density in the donors' blood did not reliably influence the incubation period in the recipient, but results were presented for only 10 patients.

Grieg and Neill (1939) found an inverse correlation between inoculum size and incubation period, but only nine patients were studied. Boyd's results on 26 patients also suggest a

negative correlation with prepatent and incubation period (Boyd 1940c).

The relationship between dose and severity of disease

P vivax

Mosquito-induced infections

A few years after starting malaria therapy James (1926) noted that the number of mosquitos employed had no influence on the severity of infection. After nearly 30 years experience of malaria therapy, Shute (1951) commented "perhaps one of the most surprising findings is that the number of sporozoites injected [in terms of mosquito bites] on a single occasion or on many different occasions does not seem appreciably to influence the severity of the disease, the duration of the attack or the gametocyte output". In other papers from this laboratory it was noted that patients infected towards the end of a batch of mosquitos - by which time the number of viable sporozoites may be reduced - sometimes got "abortive" primary attacks with only two or three days of irregular fever, or only latent infections (James et al 1936, Shute 1946a). No numerical evidence was provided for either statement.

Boyd and Stratman-Thomas (1933a) felt that the "clinical course of malaria and the possibility of spontaneous termination are related to the number of infected mosquitos applied", the duration of the initial remittent fever "being frequently proportional to the number of infected mosquitos applied." In lighter infections they felt that the patient was more likely to proceed directly to tertian fever and rigors. However in another paper that they published in the same year (1933b) the quantitative results did not support this impression. Neither the proportion of patients experiencing chills nor the proportion requiring medical intervention in the attack were related to the number of mosquitos used (Table 3.2.6). Further detail was given in the paper but the numbers in the subgroups become too small to be helpful. The results pool primary and reinfections of four strains of vivax malaria in Black and White patients, some of whom came from areas endemic for malaria.

Jerace (1934), recorded the number of mosquitos used for malaria therapy with Madagascar strain *P vivax* on 22 patients. He also recorded the time from first appearance of sporozoites in the glands until the mosquito was used. He found no association between either the number used (in the range 4-30) or the storage time, and the type of fever (quotidian or tertian), the number of spikes of fever, or whether the infection resolved spontaneously.

In Boyd and Kitchen's study (1938) where groups of three patients were bitten consecutively by the same group of mosquitos, those bitten on the first application had, on average, longer lasting attacks than those bitten on the third application. Among those bitten on the first application the duration of the attack was not related to the number of mosquitos used. It is not clear if all of the attacks recovered spontaneously.

In Boyd's studies where he graded the load in the mosquitos according to the number of oocysts he looked at various measures of severity. In the first series (which explicitly referred to pooled results with more than one strain and primary and subsequent inoculations) the proportion of patients experiencing chills, the proportion requiring induced termination of the infection, and the proportion showing "renewed activity" all increased with the grade of infection of the mosquito lots used. The proportion showing tertian fever during the infection decreased with grade (Table 3.2.7), (Boyd & Stratman-Thomas 1933b).

In a later series (Boyd 1940b), which used a different grading system in which Grade A lots had heavier infections than Grade C, there was little difference between the two groups in the proportion of cases terminating spontaneously: 121/187 (65%) for A and 55/75 (73%) for C. Considering only those whose infections terminated spontaneously, there was a positive correlation between the duration of clinical illness and the number of mosquitos employed (Grade A group: r = 0.14, 95% CI 0.03 to 0.26; Grade C group: r = 0.22, 95% CI 0.04 to 0.39). Patients from group C tended to have fewer paroxysms of fever over 104°F, but the confidence intervals were wide. Fewer patients in group C had recurrences (33% compared with 42% in group A), but again this was not significant. These results are probably the pooled experience from more than one strain and from primary and subsequent inoculations.

Among 13 volunteers bitten by one mosquito, the duration of the patent parasitaemia was related to the salivary gland sporozoite load of the mosquito. However all the attacks were interrupted by a dose of chloroquine (Coatney et al 1950b). Sioli et al (1939) noted no relation between the number of bites by mosquitos carrying the Madagascar strain and the character of the infection among 31 patients. Some of the patients had had previous inoculations.

Direct sporozoite inoculation

There is no direct evidence relating dose to outcome in sporozoite inoculations for *P vivax*. In Hamilton Fairley's study (1947), using subinoculation from one volunteer to another shortly after the first bite, the parasite counts at the time treatment was started were lower in the

recipients than in the donors. He commented that treatment was "equally necessary on medical grounds" in two of the recipients, despite the relatively low counts. For the recipient with the lowest count treatment was started for non-medical reasons.

Trophozoite-induced infection

Polayes and Derby (1934) noted that there were no differences in the occurrence of initial remittent fever between patients who received compatible or incompatible blood in a group of 121 neurosyphilis patients injected intravenously with 5ml donor blood. No other comparisons in the severity of the outcome were made. The species of malaria was not specified.

Wethmar (1927) found that those who received compatible blood were less likely to have tertian fever (Table 3.2.8). Wendleberger (1930) found similar trends: quotidian fever was much more frequent in those receiving compatible blood, and pure tertian fever was found in almost all of those who received incompatible blood.

Hoch et al (1940) diluted the donors' blood before intravenous injection to obtain small numbers of parasites. Using calculated challenges of 100 to 1000 parasites of the McCoy strain, they reported no correlation between dose and the character of the infection. Analysis of their data for 21 successful inoculations with a known dose, shows no correlation between dose and the number of paroxysms, but after lower doses a larger proportion of patients had a spontaneous recovery. Blood groups were not mentioned in this report and some of the patients may have had previous malaria therapy.

Boyd (1940a) gave the results from primary inoculations in 31 patients inoculated intravenously. Several strains were used, the blood was not cross-matched, and in one third of the patients the attacks were interrupted by treatment. Following inocula of less than 6 million trophozoites the geometric mean duration of the clinical attack was 12.5 days (95% CI 7.2 to 22.0), compared to 16.4 days (95% CI 11.3 to 24.0) following inocula of 6 million or more trophozoites .

Kaplan et al (1946b) reported measures of outcome in relation to the size of the trophozoite inoculum given intravenously. It is not clear whether a single strain was used. Among 121 patients who had not had previous malaria the proportion experiencing tertian fever decreased, and the duration of remittent fever increased with increasing dose (Table 3.2.9).

P falciparum

Mosquito-induced infections

For falciparum malaria, James et al (1932) felt that the effect of dose on the infection was "considerably greater ...in general the primary attacks of cases infected by the bites of many infected mosquitos are more difficult to control and are of longer duration than of cases infected by the bites of only one or two insects". No numeric data were given, but the number of cases was said to be small.

Boyd and Kitchen (1937a) found that the duration of illness did not appear to depend on the number of mosquitos used. This paper probably refers to more than one strain and to both primary and subsequent inoculations, but it is not stated. Interpretation is difficult as 49 of the 59 patients received treatment with quinine during the acute attack.

Direct sporozoite inoculation

Covell et al (1949) reported results of intravenous inoculations with known sporozoite doses in 30 patients receiving malaria therapy. The authors commented that there was no evidence of an effect of dose on the severity of the clinical attack, though no figures are given. The patients were all treated early in the infection, with a variety of different treatments.

P malariae

There is very little information linking dose and severity for *P malariae*. Ciuca et al (1943) had experience with 293 patients, but only presented the results for ten; they found that the parasite density in the donors' blood did not influence the parasite density in the recipient. In one of Boyd's studies (1940c) 3/7 of the infections induced by doses of less than 5 million trophozoites required treatment compared with 14/19 of those produced by higher doses.

The relationship between dose, the response to treatment and relapse rates

P vivax

Mosquito-induced infections

Coatney et al (1950b), using the Chesson strain of *P vivax*, assessed the response to suppression by different doses of chloroquine in prisoners bitten by mosquitos. Suppression

was satisfactory in 4/9 receiving 30 bites and in 8/9 receiving only three bites. The attacks which occurred after the chloroquine was stopped were, on average later and fewer in those who had received three bites.

Alving et al (1948), using the same strain in volunteers, found higher relapse rates after treatment in those who had been bitten by 80 mosquitos than in those bitten by 10 (Table 3.2.10).

Tiburskaja et al (1968) compared relapse rates with the number of mosquitos used to treat patients in psychoneurological hospitals in Moscow. The results quoted pool their experience with 10 different strains over 25 years and are divided according to the initial incubation period. Among 141 patients bitten by between 1 and 12 mosquitos who developed malaria within the usual incubation period, the number of mosquitos used was not related to the number of relapses. Among the 95 who only developed malaria after a latent period of over 6 months, those bitten by more mosquitos were more likely to have had a relapse within the follow up period which ranged from 8 months to 3 years (Table 3.2.11). It is not clear if the follow up period was similar in the different groups.

In one series from Coatney's group (Coatney et al 1950b) each prisoner volunteer was bitten by a single mosquito carrying the Chesson strain and each attack of malaria was interrupted by a single dose of 0.3g of chloroquine. All the volunteers were followed for seven months. The 6 volunteers who were bitten by a mosquito scored as 2+, according to the number of sporozoites in the glands, had a mean of 3.0 attacks (95% CI 1.5 to 4.5). The 7 who were bitten by a mosquito scoring 4+ had a mean of 4.7 attacks (95% CI 3.6 to 5.9).

Using the St Elizabeth strain of vivax malaria also in prisoner volunteers, Coatney et al (1950a) found an inverse relationship between inoculum size and the time of occurrence of "late activity" (latent infections or recurrences occurring after four months). In these experiments the inoculum was measured as the sum of pluses from the different mosquitos used. Overall, 177 of the 198 subjects experienced late parasitaemia, and a further 8 were lost from observation. The volunteers had received a variety of different treatments, both before inoculation and during the initial infection. The results for the 153 subjects for whom accurate onset dates were available are given in Table 3.2.12. It is not possible to separate the latent infections from the recurrences.

Direct sporozoite inoculation

In Hamilton Fairley's subinoculation experiments (1947), where the recipients were inoculated with blood from the donor shortly after the donor was bitten, all three donors but only one of the three recipients got secondary attacks.

P falciparum

Covell et al (1949), in reporting the results of 30 intravenous inoculations of sporozoites for malaria therapy, commented that there was no relationship between dose and the frequency of relapse, but no figures were given. The patients were given various different treatments early in the course of the infection.

The relationship between incubation period and severity of disease

P vivax

Several authors have compared the incubation or prepatent periods with the outcome of the disease using various measures of severity. The prepatent period has been referred to as a measure of the "severity" of the infection (Alving et al 1948, Jones et al 1948), and when associations are found between the prepatent or incubation period and other outcome measures they are thought to be due to the effect of inoculum size (Boyd & Kitchen 1938, Craige et al 1947). However, the confounding effects of variations in patient immunity and innate susceptibility must also be considered.

For mosquito-induced infection, Boyd found that shorter incubation periods were associated with longer attacks (Boyd & Kitchen 1938, Boyd 1940b). However neither paper gave any information on the strains used, the second paper gave no information on immunity, and the first paper pooled results from immunes and non-immunes. Confounding by strain and immune status is likely in these results. Incubation period was noted not to be associated with recurrences, but no evidence was presented (Boyd & Kitchen 1937d).

A Chicago group carried out a series of studies using Chesson strain vivax malaria in non-immune prison inmates in Stateville penitentiary. They found that the relapse rate after treatment was higher in those with shorter prepatent periods (Craige et al 1947, Alving et al 1948, Jones et al 1948). Also, those with shorter prepatent periods were more likely to have

more than one relapse and had a higher proportion of early relapses (Craige et al 1947). Tiburskaja et al (1968) found that patients with initial latent infections had fewer relapses, but it appears that this group had a shorter period of follow up after the first clinical infection.

Among psychotic patients receiving trophozoite-induced infections, the Chicago group found that shorter incubation periods were associated with a longer duration of fever and a greater number of paroxysms (Whorton et al 1947b). Jerace (1934) gave results for 22 mosquito-induced and 30 trophozoite-induced infections. For trophozoite-induced but not mosquito-induced infections the incubation period was negatively correlated with the number of spikes of fever. For both types there was a tendency for longer incubation periods to be associated with spontaneous recovery and with tertian rather than quotidian fever, but these associations were not significant at the 5% level. In Hoch et al's studies (1940) in which low doses of McCoy strain trophozoites were used, incubation period was not associated with the number of paroxysms or spontaneous recovery among 21 ill patients. No information on prior inoculations was given.

Shute (1952) stated without supporting evidence that when the incubation period was only 2-3 days, such as after the injection of large numbers of parasites intravenously, then the subsequent fever was more severe than when the incubation period is 9-14 days. (In the same article he stated that the incubation period in mosquito-induced malaria is not influenced by the number of parasites, despite evidence, including some from his own laboratory, to the contrary.)

P falciparum

One anecdotal report commented that there was no relationship between incubation period and severity of disease in malaria therapy patients (Raffaele 1951).

INDUCED MALARIA IN ANIMALS

Animal experiments have the advantage that infections are allowed to proceed further and invasive measurements of severity can be made. They have the disadvantage that the parasitic relationships studied are not generally those that occur in nature so their relevance to host-parasite relationships in human malaria is questionable. The main groups of animals used are birds, monkeys, and, more recently, rodents. Most of the experiments use blood inoculation

and therefore trophozoite-induced infections. Blood grouping is not mentioned.

Bird malaria

GH Boyd (1925) showed a clear negative correlation between the infecting dose and the "incubation period" (probably the prepatent period) using *P praecox*. He also found a correlation between the dose and the height of the peak parasitaemia, but no relationship between the dose and the length of the period of rise of parasite counts.

For adult ducks inoculated with *P lophurae* there was no clear dose-response relationship in terms of the peak parasitaemia or the proportion of fatal infections, although the period of rise in parasite numbers decreased with higher doses (Hewitt 1942). However, for young ducks, higher doses were associated with shorter prepatent periods, decreased time to the peak parasitaemia, increased height of peak parasitaemia, more profound anaemia, a higher proportion of deaths, and decreased time to death (Hewitt et al 1942). Wolfson (1945), using *P relictum* in young ducks, also found higher peak parasitaemias and lower haemoglobin concentrations with increased doses.

Greenberg et al (1950) gave intravenous sporozoites of *P gallinaceum* to chicks. At lower doses the prepatent periods were longer and more birds survived following treatment.

Monkey malaria

Rhesus monkeys were the usual species used for experiments. They show exquisite sensitivity to the monkey malaria *P knowlesi*: one parasite can cause a fatal infection. As the dose increases the time to death decreases. The variation is in the prepatent period, the time from first appearance of the parasite being approximately constant (Coggeshall & Eaton 1938). Using trophozoite-induced *P cyanomolgi* infections, Wolfson and Winter (1946) again found the prepatent period to be dose-related. Peak parasitaemia also increased with dose, but there was no dose-relationship with the time taken to reach the peak, nor with the duration of the infection.

Also using *P cyanomolgi* in rhesus monkeys, Schmidt et al (1982) found an inverse relationship between dose and prepatent period for both trophozoite and sporozoite-induced infections. In trophozoite-induced infections the time from patency to the peak and the height

of the peak were independent of dose in the range 10^5 to 10^7. For sporozoite-induced infections the duration of the attack appeared to increase with inoculum size, but with wide variation.

By externalising the spleen, Coggeshall (1937) was able to measure splenomegaly in infected rhesus monkeys. With *P knowlesi* greater splenic enlargement was noted at lower doses, that is, when death was more delayed. For *P inui* no relationship between dose and spleen size was noted, but only six monkeys were used for the experiment and the dose range was small.

Rodent malaria

Most of the experiments have been carried out using *P berghei*. For both trophozoite and sporozoite-induced infections, lower doses have consistently been shown to be related to longer prepatent periods (Fabiani & Orfila 1954, Arcoleo & Carrescia 1955, Vanderberg et al 1968, Nussenzweig et al 1966) or longer latent periods to a given level of parasitaemia (Sengers et al 1971a,b, Warhurst & Folwell 1968).

In most experiments almost all of the animals with patent infections died (Nussenzweig et al 1966, Sengers et al 1971b). Ferraroni (1983) found that the proportion that died was dose-related. The time to death showed a variable relationship with dose. In some systems the time to death was bimodal, and dose did not influence whether the mice had slow or rapid deaths (Arcoleo & Carrescia 1955, Sengers et al 1971b). However, within each group (slow or rapid) the time to death was dose-related (Sengers 1971b). In other systems there was a simpler relationship between increasing dose and decreasing survival times (Nussenzweig et al 1966, Vanderberg et al 1968). In contrast to the monkey experiments, this appeared to depend on a decreasing period of patent parasitaemia as well as shortened prepatent periods with increasing dose (Nussenzweig et al 1966).

Using *P vinckei*, dose was again found to be negatively correlated with survival time (Cox 1966). One small experiment failed to show this but used just two different high doses (Yoeli et al 1966).

CONCLUSIONS

An inverse relationship between dose and prepatent or incubation period is found in both animal experiments and human induced malaria. A relationship with severity is suggested by the animal experiments but hard to demonstrate in the published human data. A few papers indicate a relationship, but much of the information comes from small studies and is often presented with incomplete information, and is marred by pooling of results from different strains, and immune and non-immune patients. The most consistent evidence is of a relationship between prepatent or incubation period and severity, but this could be due to confounding by host susceptibility or, in some studies, immunity. Some of these problems are overcome in the following analyses of induced malaria data.

Table 3.2.1. Experimental infections with Chesson strain *P vivax* (from Coatney et al 1950b).

No. of mosquitos	n	Prepatent period (days) mean (95% CI)	Incubation period (days) mean (95% CI)
10	96	11.77 (11.48-12.06)	12.16 (11.79-12.53)
1	15	13.93 (13.18-14.68)	14.00 (13.44-14.56)

Table 3.2.2. Influence of the compatibility of blood group and inoculum size on the incubation period of Chesson strain *P vivax* (from Whorton et al 1947a).

Inoculum (in millions of parasites)	n	Incubation period (days) Mean (range)
1) Compatible blood		
< 1	9	5.4 (3 - 11)
1 - 9	6	2.2 (2 - 4)
10 - 99	29	2.0 (< 1 - 5)
100 - 999	17	0.9 (< 1 - 3)
≥ 1000	5	0.5 (< 1)
2) Incompatible blood		
< 1	4	8.8 (8 -10)
1 - 9	6	8.3 (6 -12)
10 - 99	22	4.2 (< 1 - 7)
100 - 999	23	3.7 (< 1 - 7)

Table 3.2.3. Days to first detection of parasites in the recipient compared with the number of mosquitos used for *P falciparum*. Geometric means and 95% confidence intervals are shown. (From Boyd and Kitchen 1937e)

No. of mosquitos	Coker strain		Long strain		Overall (5 strains)	
	n	mean	n	mean	n	mean
1	5	12.9 (10.7-15.5)			8	10.6 (8.1-13.8)
2-5	17	11.6 (10.4-13.0)	2	11.0 (11.0-11.0)	23	11.4 (10.5-12.4)
6-10	18	12.5 (10.9-14.4)	28	10.8 (10.4-11.1)	46	11.4 (10.7-12.1)
11-20	2	13.5 (8.4-21.6)	11	9.9 (9.3-10.5)	17	11.0 (9.9-12.3)

Table 3.2.4. Prepatent and incubation periods in days by the number of mosquito bites for three different strains of *P falciparum* (from Jeffrey et al 1959).

No. of mosquitos	Panama strain			McLendon strain			Santee-cooper strain		
	n	median	(range)	n	median	(range)	n	median	(range)
Prepatent period									
1-10	15	11	(9-16)	14	13	(10-29)	7	10	(9-13)
11-20	31	10	(7-16)	11	12	(10-15)	-		
21+	11	9	(8-15)	8	12	(9-14)	8	9	(7-13)
Incubation period									
1-10	15	13	(9-19)	14	14	(9-32)	7	13	(7-14)
11-20	31	13	(9-17)	11	13	(11-15)	-		
21+	11	11	(7-17)	8	13	(11-21)	8	10.5	(7-16)

Table 3.2.5. Relationship between sporozoite dose, prepatent period and incubation period in falciparum malaria (from Covell et al 1949).

Sporozoite dose (in thousands)	Proportion of patients in each category	
	Prepatent period ≤ 7 days	Incubation period ≤ 7 days
< 100	5/11	7/11
100-200	6/9	5/9
≥ 200	10/10	10/10

Table 3.2.6. The effect of mosquito number on the occurrence of "chills" and the need to induce termination of the attack in *P vivax*. Pooled results of 4 strains and primary and reinfections (from Boyd & Stratman-Thomas 1933b).

No. mosquitos	Chills	Termination induced
1	15/23 (65%)	11/23 (48%)
2	7/7 (100%)	2/7 (29%)
3	7/8 (88%)	3/8 (38%)
≥ 4	9/12 (75%)	3/12 (25%)

Table 3.2.7. The proportion of patients with various outcomes by grade of infection of the mosquito lots used. Pooled results from more than one strain of *P vivax* and primary and subsequent inoculations (from Boyd & Stratman-Thomas 1933b).

Grade	Chills	Requiring termination	Tertian fever	Renewed activity
Poor	5/10 (50%)	1/10 (10%)	6/10 (60%)	3/10 (30%)
Fair	8/11 (73%)	4/11 (36%)	5/11 (45%)	6/11 (55%)
Good	25/29 (86%)	14/29 (48%)	7/29 (24%)	16/29 (55%)
χ^2 trend	5.23	4.37	4.57	1.5
p	0.02	0.04	0.03	0.2

Table 3.2.8. Outcome in 65 patients inoculated with vivax malaria in relation to compatibility of the blood inoculated (from Wethmar 1927).

	Intravenous inoculation		Intracutaneous inoculation	
	Compatible	Incompatible	Compatible	Incompatible
Mean incubation period (days)	4.5	8.2	9.8	15.1
% with each type of fever:				
Quotidian	46%	6%	13%	8%
Tertian	27%	82%	67%	92%
Mixed	27%	12%	20%	0%

Table 3.3.9. Relationship between inoculum size and type of fever in trophozoite-induced vivax malaria (from Kaplan et al 1946b).

Inoculum (parasite nos in millions)	n	Tertian cycles	Days of remittent fever Mean (95% CI)
1	28	12/28 (43%)	1.64 (1.00-2.28)
2 - 5	28	9/28 (32%)	2.50 (1.90-3.10)
6 - 25	29	9/29 (31%)	2.34 (1.80-2.88)
26 - 150	36	9/36 (25%)	2.86 (2.29-3.43)

χ^2 trend in tertian cycles = 2.1, p = 0.1

Table 3.2.10. Relapse rates following treatment in experimental vivax malaria using the Chesson strain. Relapse rate is the ratio of the number who relapse to the number of individuals treated. (From Alving et al 1948).

No. of mosquito bites	Pentaquine + quinine	Quinine alone	Other suppressive drugs
10	4/43	10/11	52/57
80[1]	4/4	2/2	4/4

[1] or intracutaneous injection of salivary glands from 45-75 mosquitos

Table 3.2.11. Number of patients suffering different numbers of relapses following an initial latent infection with vivax malaria, related to the number of mosquitos used to induce an infection. Pooled experience with 7 strains (from Tiburskaja et al 1968).

No. mosquitos	n	No.(%) suffering each number of relapses					
		0		1		2	
1	52	36	(69%)	13	(25%)	3	(6%)
2	25	16	(64%)	7	(28%)	2	(8%)
3-10	18	6	(33%)	6	(33%)	6	(33%)

(χ^2 trend with increasing mosquito number comparing relapses vs no relapses = 11.5, $p < 0.001$)

Table 3.2.12. Relationship between the infective inoculum and the time to the onset of late parasitaemia in mosquito-induced St Elizabeth strain vivax malaria (from Coatney et al 1950a).

Inoculum (sum of pluses)	n	Days from mosquito bites to first day of late parasitaemia. Mean (95% CI)
1 - 10	6	292 (257-327)
11 - 20	34	297 (288-306)
21 - 30	63	285 (279-291)
31 - 40	46	267 (254-280)
41 - 50	1	276
51 - 60	3	256[1]

[1] standard error not given

ANALYSIS OF DATA FROM THERAPEUTIC MALARIA RECORDS

PLASMODIUM VIVAX

MATERIALS AND METHODS

The raw data

Malaria therapy for neurosyphilis was carried out at the Horton hospital, Epsom, Surrey, from 1923 to the 1960s, conducted from the laboratory which became the Malaria Reference Laboratory for the UK (Covell & Nicol 1951). Malaria was induced by mosquito bite, blood inoculation, or direct injection of sporozoites. Information regarding these infections exists in three sources: two sets of books compiled at Horton, and the original notes of the patients who received the therapy. One set of books is now stored in the library at the London School of Hygiene and Tropical Medicine. These books are arranged by episode of induced malaria and give the name of the patient, the date (or dates) of the therapy, the strain of malaria used, the type of malaria therapy given, a measure of dose depending on the type, the date the fever started, the date parasites were first found in the blood, the type of fever, the parasite counts on each day, and details of any treatment given. There are no temperature charts or details of the height of the fever, and, after the early years, no descriptions of the severity of the illness. Previous episodes of malaria therapy are sometimes mentioned, and the dates, treatment and parasite counts for any relapses are given (Figure 3.3.1).

A second set of books is held at the library of the Royal College of Physicians. These books are complementary to the first, referring to the same patients and being arranged by patient rather than by episode of malaria therapy. A full record of any previous malaria therapy is therefore more easily obtained from them. The emphasis in these books is on the syphilis rather than the malaria, but they contain further details of the fever and subjective descriptions of the illness. They provide personal details such as sex, age and nationality, and details of any foreign travel including mention of any previous natural malaria. The date of transfer, discharge or death of the patient is usually given. These books stop in the 1950s, but contain details of around 2500 patients (Figure 3.3.2).

The Horton hospital still exists as a mental hospital. About fifty sets of notes that referred to patients who had had malaria therapy were identified among those kept in the Medical Records department. Unfortunately this department only maintains the records of patients who were in the hospital within recent years. Older records, while kept, are not in any order and are difficult to extract. The notes that were extracted contained similar details to those obtained from the books at the College (Fig 3.3.3). No complete temperature charts were found. Since these notes did not add any information and since many of the patients were transferred to other hospitals, no effort was made to trace further notes.

The Madagascar strain of vivax malaria

About half of the malaria therapy carried out at Horton used this one strain of malaria. First isolated in 1925 (Figure 3.3.4) it was maintained by repeated passage through man and mosquito until the end of malaria therapy in the 1960s. In 1933 the strain was transferred to Holland, where it continued to be maintained in man and mosquito, while therapy was tried with another strain. In 1936 the strain was returned to the UK (Shute 1946b). Before 1933 every batch of mosquitos was infected from a mosquito-infected patient, but after 1936 a shortage of patients meant that some batches were infected from blood-infected patients. No loss in virulence was noted (Shute 1952). Between 1925-6 and 1930-2, however, gametocyte production by the strain changed: gametocytes were found earlier and in larger numbers in the later period (James et al 1936).

Typical infections with the Madagascar strain were described by Shute (1952):

> "1) Initial fever usually lasts 3-5 days and is irregular, the temperature seldom rising above 102°F, and it may not return to normal throughout this period.
>
> 2) Developed stage. - Fever is quotidian [in over 80% of primary cases], temperatures of 104°F and 105°F are usual, rigors may or may not occur, and after 5 or 6 attacks the patient feels rather exhausted.
>
> 3) Terminating stage. - After ten or more uninterrupted attacks of fever the tendency is towards spontaneous recovery, but this depends on the amount of resistance which the patient has built up as the result of his previous attacks. The first indication of spontaneous recovery may be the fever's sudden change from quotidian to classical tertian; but this does not always happen, and patients who are having quotidian fever may recover spontaneously, although parasites may be plentiful in the blood.
>
> 4) Chronic stage. - After a spontaneous recovery, either from the primary attack or from a relapse, parasites may be quite numerous in the blood and may continue to be so for several weeks unless antimalarial drugs are given."

Data extraction and entry

I extracted and entered all the data myself, directly on to the computer, using Epi Info. Records from the different sources were collated using the name, the date and the strain of malaria. Since the data were entered directly and the process of extracting the data was time-consuming, double entry was not feasible. The decision to enter it in this way was taken so as to avoid the transcription errors which would have occurred if the data had been extracted as a separate step. Some internal checks were possible within the data, such as range checks, and consistency checks.

The data from all patients who had received malaria therapy with Madagascar strain vivax malaria were entered unless they had received prophylactic treatment (ie treatment in the period from two weeks before inoculation to the start of the fever or parasitaemia, whichever was first) or had received another strain or species of malaria concurrently. The books which referred to the episodes of malaria were regarded as the first set, and the books describing the patients were matched to them. Any episode appearing only in the second set would therefore not be included, but in practice this did not occur.

Many of the patients were infected with malaria on more than one day so a rule was made to define success and failure in episodes where malaria was given on several occasions some time apart: if patent parasitaemia or fever did not develop within 30 days of the dose the episode was called a failure. In some episodes further malaria was given during the illness. This was recorded.

Mosquito-induced malaria

The mosquitos used were almost always *A maculipennis* var. *atroparvus*. At first they were collected in the wild, mainly from pigsties, but from 1933 a breeding colony was established at Horton (Shute 1951). The mosquitos were infected by feeding on a patient who had been shown to have gametocytes in the blood. The mosquitos which had fed were identified by examination for bright red blood and were separated from the rest. A few mosquitos from the batch were dissected each day from the third day after feeding to ascertain the percentage infection of the batch and the time at which sporozoites were first found in the glands (Fig 3.3.5) (Shute 1951). Several of the early books refer to storage of mosquitos containing mature sporozoites in ice chests (Figure 3.3.6), and it seems likely that this was usually the case (as in

the USA (Boyd & Stratman-Thomas 1934, 1936)). I have not been able to find published documentation of this.

The mosquitos used to bite a patient were placed in a jar covered in netting and this was applied to the patient's thigh (Nicol 1927). Whether the skin was cleaned first or warmed with a hot water bottle seems to have varied over time (Nicol 1927, Shute 1952). From the seventh day after infection four-hourly temperature charts were started, and temperatures were recorded every 15-30 minutes during attacks to ensure that the height of the peak was correctly measured (Nicol 1927, Shute 1952). Cold sponging was begun when the temperature reached 105°F (Nicol 1927). Thin blood films, stained with Leishman's stain, were examined daily; thick blood films were rarely used (Nicol 1927, Shute 1952). Parasite counts were recorded per 25 high power fields, or per 100 high per fields at the beginning and end of the infection. Nicol (1927) reports that these fields were those seen using a 1/12-inch oil immersion lens and a No. 2 eye-piece. The later account (Shute 1952) describes the same lens and a No. 6 eyepiece. In this system there are about 400 red blood cells per field (Shute 1952).

The attacks were often modified in their early stages by a single or a few doses of treatment. Quinine 0.3g was generally given in the early years, and thiobismol 0.2g from the 1940s (Nicol 1927, Whelen & Shute 1943, Shute 1952). After thiobismol the fever was interrupted for a day, and after quinine for about 14 days (Nicol 1927, Shute 1952). A primary objective of the modifying treatment was to convert quotidian fever into tertian fever which was felt to be less exhausting for the patients (Whelan & Shute 1943). Treatment was given if the parasite count exceeded 1-2 per high power field (Nicol 1927, Shute 1952), or if the patient experienced "persistent vomiting, fainting or collapse during the paroxysm, cyanosis, seizures, undue restlessness ..albuminuria [or] jaundice". In reaching a decision on whether to give treatment, and whether it should be full or modifying, account was taken of "the clinical condition of the patient, the duration of the paroxysms, the maximum temperature reached" and whether the fever was quotidian or tertian (Nicol 1927). Since the therapeutic effect of malaria was thought to be due to the fever and to the "parasite and its toxins in the patient's system" the fever was allowed to run for ten or more paroxysms if possible (James 1931).

Measures of dose (exposure variables)

For each episode the number of mosquitos that bit the patient and the date or dates on which they were used were recorded. Using information from the dissections carried out on the batch, the proportion of infected mosquitos in the batch was found. An estimate of the number of

infected mosquitos was calculated by multiplying this proportion by the number of mosquitos used. The true number of infected mosquitos is unknown. The number of mosquitos dissected in each batch varied from eight to over one hundred, and the batch used was not always recorded, particularly in the earlier years.

The date on which sporozoites were first found in the glands of the mosquitos of the batch was recorded. The subsequent storage time until the mosquitos were used was calculated, since it had previously been shown to influence the attack rate (Boyd & Kitchen 1937b). In the analysis this storage time has been used as an exposure variable (as a possible marker of the number of viable sporozoites) and as a possible confounder when looking for associations with mosquito number.

Since prepatent period (the time from inoculation to patent parasitaemia) and incubation period (the time from inoculation to symptoms, taken as fever in this data set) are inversely related to dose, they can be considered as indirect measures of dose. This relationship is explored further in the data. For reasons addressed in the discussion, they may provide a better marker of the number of viable sporozoites received - the true dose - than the other measures of exposure.

Outcome variables

From the first set of books the outcome variables available were the peak parasitaemia reached (pretreatment and overall), the type of fever (which was also given in the second set) and the treatments given. The second set of books recorded the peak temperature reached and the number of peaks over 103°F. When modifying treatment was given, the number of peaks before and after the treatment were sometimes recorded separately. The type of fever was usually recorded as "quotidian" or "tertian" or "irregular" or combinations of these. Again it was sometimes possible to distinguish the pretreatment fever type. The second set of books contained some descriptions of the symptoms suffered. These became less common over time. Although anaemia was sometimes noted, other patients were referred to as looking pale or very pale, and it is difficult to know how to interpret this. Only two subjective descriptions from the books have been used: "very ill" and "spontaneous recovery". Deaths were recorded in both sets of books, but the cause of death was often unclear. For this analysis, death occurring during the parasitaemia was used as the outcome variable.

Both sets of books recorded relapses, although the record appeared to be more complete for later relapses in the second set. Since the books did not appear to have a strict definition of a

relapse to distinguish it from a continuation of the same infection, a relapse was defined for the purposes of this study as a recurrence occurring after 20 days without patent parasitaemia. The time of loss to follow up was found in the second set of books and was taken as the date of transfer away from the Horton hospital. (The notes often continued after this point if the patient was in one of the adjoining hospitals, but this period was not included so that the care during follow up and the chance of relapses being noticed were more comparable).

The outcome variables used are listed below. Those relating to parasitaemia and fever were recorded both pretreatment and overall.

1) Relating to the parasitaemia:

- The peak parasitaemia, as parasites per 100 high power fields (hpf)

2) Relating to fever:

- The peak temperature reached.
- The number of peaks over 103°F.
- The number of peaks over 103°F as a proportion of the number of days between patent parasitaemia and treatment.
- Whether the fever included an episode or episodes of tertian fever.
- Whether the fever was predominantly tertian (ie pure tertian or tertian with some irregular fever).

3) Based on descriptions in the second books:

- Whether the patient was said to be very ill during the malaria
- Whether the patient was noted to have made a spontaneous recovery. The published reports suggest that this referred to the fever and not the parasitaemia (Nicol 1927, Shute 1952).
- Whether death occurred during the period of patent parasitaemia

4) Relating to treatment:

- The time to any drug treatment
- The time to the full course of drug treatment
- The time to the first dose of modifying treatment
- Whether modifying treatment was given
- The number of doses of modifying treatment given
- Whether full treatment was given
- Whether parasitaemia ended before any treatment was given
- Whether parasitaemia ended before full treatment was given

5) Relating to relapses:

- Whether any relapses occurred, and the number and timing of relapses

Confounding variables

Some of the variables already mentioned were considered as possible confounders in certain situations. For example, the use of modifying treatment might confound associations involving the timing of full treatment. Other possible confounders for which information was available were:

- The date of the malaria therapy
- The sex of the patient
- The age of the patient
- Whether the patient had had any previous malaria therapy and whether it was with the same strain, same type or different type of malaria, the timing of this therapy and its outcome.
- Whether the patient had ever had natural malaria
- Whether the patient had ever travelled to a malarial area. If the patient was stated to have been in the army or at sea but no areas of travel were listed this was recorded as unknown.

Trophozoite-induced malaria

Trophozoite-induced infection was used throughout the period of malaria therapy and more frequently in the later years. Whole blood was used and the usual practice was intramuscular injection just below the scapula (Shute 1952). The blood was generally taken a few hours after an attack of malaria and, for patients infected by subinoculation at Horton, it appears that the blood was used immediately and was therefore not heparinised (Shute 1952). Blood grouping of the patients was not mentioned and no blood groups are recorded in the books or notes, so it seems unlikely that the blood was cross-matched. The care and subsequent treatment of the patient was the same as for mosquito-induced malaria.

Measures of dose (exposure variables)

The first set of books recorded the date of injection, the amount of blood used, the name of the patient from whom the blood was taken, and sometimes the route of infection. From the donor's record, the parasite count on that day was determined (in terms of parasites per 100 high power fields). The "dose" is calculated as the volume multiplied by the parasite count. This assumes that the parasite count was recorded at approximately the same time as the blood was taken. Since one high power field contains about 400 red blood cells (Shute 1952) and assuming a red blood cell count of 4×10^9/ml, to estimate the actual number of parasites inoculated the calculated dose is multiplied by 100000. As in mosquito-induced infection, the prepatent period and incubation period have been used as alternative, indirect measures of

dose.

Outcome and confounding variables

The same outcome and confounding variables were used as for mosquito-induced infection except that storage time did not need to be considered, and true relapses do not occur.

Sporozoite-induced malaria

James et al (1927) described a technique for extracting sporozoites. The salivary glands of one or more infected mosquitos were dissected out into human serum or Locke's fluid. After confirming the presence of sporozoites a cover-glass was put over the glands and pressed with a needle to squeeze out the sporozoites so that they floated freely. The serum and sporozoites were drawn up together in a syringe for injection. Ten years later Shute (1937) described how the technique had been improved to determine the number of sporozoites injected. In this publication the medium was Locke's fluid and the importance of sterility of the fluid and glass slide and cover-slip were stressed. A drop of blood was added to the fluid to aid focusing and help enumeration of the sporozoites. The mixture of fluid and sporozoites was made up to 1ml and a count was made on 0.01ml after staining. Shute noted that sporozoites did not live for more than a few hours in Locke's fluid.

Measures of dose (exposure variables)

For many of the patients an estimate of the number of sporozoites injected was available. As for mosquito-induced malaria the storage time of the mosquitos was calculated. In a few records in the first set of books comments about the state or treatment of the mosquitos or sporozoites were recorded which might have affected the outcome. These were:

Comment	No. affected
Mosquitos over frozen	5
Sporozoites from oocysts or stomach	7
Sporozoites from coelomic fluid on legs	2
Sporozoites looked shrunken or dead	3
Sporozoites incubated at 37°C	4
Sporozoites centrifuged	1
Sporozoites stored 48/72 hours	2

Of these 24 inoculations only 7 (29.2%) were successful, compared with 105/114 (92.1%) of the others, and those that were successful had longer prepatent periods, suggesting a low number of viable sporozoites. This group of patients has been excluded in analyses using

sporozoite number or storage time, but have been retained in analyses using the prepatent or incubation period. The route of injection was recorded for 60 patients overall, of whom all but one received intravenous inoculations.

Outcome and confounding variables

The same outcome and confounding variables were used as for mosquito-induced infection.

Strategy of analysis

The analysis was carried out using Epi Info and Egret. The continuous variables were inspected to see if they were normally distributed or better described by another distribution such as the log normal distribution. For some of the analyses the continuous variables were divided into groups. This was done after consideration of several factors: the statistical advantage of having approximately equal numbers in each group; any natural discontinuities in the distribution (such as in the date of therapy); and the general character of the distribution. To allow logistic regression to be used some of the outcome variables were redefined as binary variables using a cut off point. For example, a high temperature was defined as a fever of at least 106°F.

With the exposure variables grouped, the outcome variables were compared to them by tabulation of discrete variables and by comparing the means (or geometric means) for the continuous variables. Exposure and outcome of continuous variables were compared using scatter plots and linear regression. Confounders were allowed for using multiple linear regression, stratification and logistic regression, as appropriate. In logistic regression, where several forms of a confounding variable were available (such as year as a linear variable, or grouped in three or four groups and then treated as a linear or factored variable) the form which explained most of the deviance of the model, allowing for differences in degrees of freedom, was used.

Since many statistical tests were done for each data set due to the variety of measures of both dose and outcome, the finding of some small p values is to be expected due solely to chance, and results have to be interpreted very conservatively.

RESULTS

(The main results are summarised in Table 3.3.20.)

Mosquito-induced malaria

Information was available on 1298 episodes of mosquito-induced infection. Positive parasitaemias were recorded in 1164 and this was associated with fever in all but 7. (These 7 had all had previous malaria therapy with the Madagascar strain and had parasitaemias of 1/100 hpf or less.) Two episodes gave rise to latent infections with prepatent periods of 147 and 252 days. Other prepatent periods were between 7 and 26 days except for one of 46 days. Full details were not available for all episodes so the numbers given in the descriptions of the variables do not always add up to the full total. In particular, information from the second set of books was only available for 1156, and on batch for 1146.

In the first few years the patients were exclusively female, males only being included from the end of 1931. Overall, 46% were female. The mean age was 44 years, with a minimum of 7 and a maximum of 75. Most of the patients were treated between 1936 and 1950, with a range from 1925 to 1959. Previous malaria therapy had been given to 224, and in 166 it was with the same species of malaria. Thirty-four were known to have had previous malaria (unknown in 213) and 129 were known to have travelled to malarial areas (unknown in 446). Excluding those who had had previous malaria therapy with *P vivax* or were known to have had natural malaria leaves 1093 of whom 1039 (95%) developed fever and parasitaemia. Only those who developed fever and parasitaemia are considered further.

Records were only included in the subsequent analysis if information was available on the batch from which the mosquitos came and on the percent of infection of the batch. Records were excluded if the prepatent period was equal to or more then 30 days, if the patient had had previous malaria therapy with the same species of malaria (including unsuccessful inoculations), or had had previous natural malaria. Episodes with longer prepatent periods were excluded because of the rules made when entering the data to define success and failure in episodes where mosquitos were given on several occasions some time apart.

The initial analysis was carried out on a subset of the data relating to successful infections in which mosquitos had been used on only one day. (Five additional patients who received further

doses after the malaria had started were included in the analysis of the association of dose with prepatent and incubation periods.) There were 540 records which fulfilled the above criteria. For all of the analyses except those relating dose to prepatent and incubation periods, only episodes where no drugs were given within the first five days of patent parasitaemia were included (leaving 289 records). The cut-off at five days was chosen following comparison of the time to treatment with various other outcome measures in the whole data set for mosquito-induced malaria. Those treated within the first five days had mean peak parasitaemias which were lower than those treated later, whereas by 6 or 7 days the mean was in the same range as the rest. The mean peak fever reached full height within the first five days.

The number of mosquitos, the number of infected mosquitos, the prepatent period, the incubation period and the storage time were all approximately log normally distributed. The peak parasitaemia approximated to a log normal distribution, and the peak temperature, the number of peaks over 103°F, and the number of peaks over 103°F as a proportion of time to treatment approximated to normal distributions. Age and the year of therapy were also approximately normally distributed.

The relationship between mosquito number, storage time and prepatent and incubation periods
The prepatent period was inversely related to the number of infected mosquitos used. Linear regression, using the logs of both variables, gave:

r = -0.26 (-0.33 to -0.18)

b = -0.0739 (-0.0975 to -0.0504)

a = 1.175, F = 37.88

Using multiple linear regression, this association was found to be independent of storage time, the year of the inoculation, and the age and sex of the patient. Longer storage times were associated with longer prepatent periods. Prepatent periods were shorter in the more recent years, and increased slightly with age and were slightly longer in men than in women.

The relationships with incubation period were similar: an inverse correlation with the number of infected mosquitos which was independent of the relationships with the other variables; positive correlations with storage time and age, a negative correlation with the year of the inoculation, and longer incubation periods in men.

Using the total number of mosquitos the results were very similar for both prepatent period and incubation period. Both sets of results are summarised in Tables 3.3.1 and 3.3.2, and the

relationships with the number of infected mosquitos and the storage time are shown in Figures 3.3.7 and 3.3.8.

Results relating to parasitaemia

Peak parasitaemia before treatment and overall was compared with the various measures of exposure by examining scatter plots, comparing the geometric means of the peak parasitaemia for different groups of the exposure variables, and using linear regression.

There were no associations found between the peak parasitaemia, before treatment or overall, and any of the measures of dose - number of mosquitos, number of infected mosquitos, storage time, prepatent period or incubation period (Figure 3.3.9, Tables 3.3.3-4). No relationships were found after allowing for possible confounders (age, sex, year of inoculation, and time to treatment). A high parasitaemia was defined as a parasitaemia of at least 100 per 100 high power fields. This occurred in 60 (20.8%) patients before treatment and 71 (24.6%) overall. High parasitaemia was not associated with any of the exposure variables, before or after allowing for confounding. The peak parasitaemia before treatment and overall was higher in the earlier years and older age groups. There were no differences between the sexes.

Results relating to temperature

The peak temperatures reached before treatment and overall were compared with the exposure variables by calculating the means for the grouped exposure variables and by using linear regression. No relationships were found between the peak temperature pretreatment or overall and the number of mosquitos, the number of infected mosquitos, the prepatent period or the incubation period (Figure 3.3.10, Tables 3.3.3-4). No relationships were found after allowing for the possible confounders. The storage time was negatively associated with the pretreatment peak but not with the overall peak. This association persisted after adjusting for confounders in a multiple regression model. A high temperature was defined as a peak of at least 106°F. This was reached by 28/257 (10.9%) of patients overall and 12/160 (7.5%) before treatment. No associations were found with high temperatures occurring overall or pretreatment and any of the exposure variables. The peak temperature overall and before treatment was higher in the earlier years and in women, though this was mainly due to confounding by year. There were no associations with age.

The number of peaks over 103°F before treatment and overall were both highly dependent on the time elapsing before treatment. Time to treatment was allowed for by including a term in

the multiple regression model, by considering results in separate strata, and by creating new variables by dividing the number of peaks by the number of days elapsing before treatment. No associations were found between the number of peaks pretreatment or overall and the number of mosquitos, the number of infected mosquitos, the prepatent period or the incubation period, with or without adjusting for the time to treatment or other possible confounders (Figures 3.3.11-12, Tables 3.3.3-4). The storage time was negatively associated with the number of peaks occurring pretreatment, but not after adjusting for the time to treatment. The year of the inoculation was negatively associated with the total number of peaks, both before and after adjusting for the time to treatment, but not with the number of pretreatment peaks. The age and sex of the patients were not associated with the number of peaks.

Episodes of tertian fever occurred in 155/283 (54.8%) of the patients overall, and in 41/226 (18.1%) before treatment. Predominantly tertian fever (after the initial phase) occurred in 13/226 (5.8%) before treatment, and in 15/283 (5.3%) overall. Neither the number of mosquitos nor the number of infected mosquitos were related to the occurrence of tertian fever at any time (Table 3.3.5). The prepatent period and the incubation period were not associated with the occurrence of tertian fever overall, but were strongly associated with the occurrence of tertian fever before treatment, and also with the occurrence of predominantly tertian fever before treatment and, for prepatent period, with predominantly tertian fever overall (Table 3.3.6). Longer storage times were associated with a higher proportion of patients with tertian fever overall, but not with the other measurements of tertian fever.

Confounding was looked for by stratification and in logistic regression models, using the grouped exposure variables as linear variables. The year of the inoculation was positively associated with the occurrence of tertian fever but not with the other measures, and the time to treatment was associated with tertian fever before treatment, but not with the others. There were no associations between sex or age and tertian fever. There was no evidence of confounding in any of the associations with tertian fever, but there was an interaction with sex: the strong positive association of prepatent period and incubation period with tertian fever occurring before treatment was seen only among the men. However, for predominantly tertian fever occurring before treatment, the associations with prepatent and incubation periods were seen among the women and not the men.

Results based on descriptions

Nineteen patients were described as "very ill" and 43 were said to have made a spontaneous

recovery. There were only six deaths during malaria in this data set and they showed no particular association with any of the exposure variables. A higher proportion of patients were described as "very ill" during the earlier period, when descriptions tended to be fuller. Spontaneous recovery was noted almost entirely during the middle period of 1935 to 1945. One patient was noted to have been very ill and to have had a spontaneous recovery.

There were no associations between the number of mosquitos or the number of infected mosquitos and whether the patient was described as being very ill or having had a spontaneous recovery (Table 3.3.5). Spontaneous recovery was strongly positively associated with prepatent period and incubation period (Table 3.3.6). There was no evidence of confounding, but the trend was only seen for men, among whom 30 of the 43 cases of spontaneous recovery occurred. Longer storage times were also associated with an increased proportion undergoing spontaneous recovery, and again this was only apparent among men. The association with prepatent period was unaltered by adjusting for storage time.

Prepatent period, and to a lesser extent incubation period, were weakly, positively associated with being very ill (Table 3.3.6).

Results relating to treatment

Full treatment was given in all but 6 patients. There were no associations between receiving full treatment and any of the measures of dose. Modifying treatment was given in 186 episodes. To measure spontaneous recovery in relation to the parasitaemia, rather than in terms of fever as used in the descriptions in the notes, two new variables were defined, one which was positive if the parasitaemia ended before full treatment was given, and the other which was positive if the parasitaemia ended before any treatment. Twenty-six cases fulfilled the first criterion and nine the second. They were related to the description of spontaneous recovery as shown in Table 3.3.7.

There were no associations between the proportion of people for whom the parasitaemia ended before full treatment was started and any of the exposure variables. The parasitaemia was more likely to end before any treatment following longer prepatent and incubation periods. There was no relationship between this outcome and the other exposure variables (Tables 3.3.5-6).

Modifying treatment was given to a higher proportion of people in more recent years and they tended to get more doses and to get treated earlier. People with longer prepatent or incubation

periods were less likely to receive modifying treatment. People who were bitten by more mosquitos or, to a lesser extent, more infected mosquitos were more likely to receive modifying treatment, and these trends were more marked after allowing for the year of inoculation (Table 3.3.8). There was no relationship between the storage time and any measures of treatment.

The number of doses of modifying treatment showed a similar relationship with the exposure variables (Table 3.3.5,6 & 8). Receiving at least two doses was negatively associated with prepatent and incubation period (though this relationship was less strong after adjusting for year) and positively associated with the number of mosquitos and the number of infected mosquitos (and these trends were more marked after adjusting for year). Receiving at least three doses was not associated with incubation period, nor prepatent period after adjusting for year. It was positively related to the number of mosquitos, and to the total number of infected mosquitos after adjusting for year.

There were no associations between the timing of treatment - full, modifying or any - and the prepatent period, incubation period, number of infected mosquitos used or the storage time, at least after allowing for the year of inoculation. Patients bitten by more mosquitos tended to receive earlier treatment, but this depended on whether modifying treatment was given, and there was no trend in the timing of modifying treatment (Figure 3.3.13).

Results relating to relapses

The length of follow up was very variable and often brief, so assessment of relapse rates has to take this into account. (The wards appear to have been cleared at the outbreak of the war by transferring patients to other hospitals, and during the war, although malaria therapy continued, patients were often admitted only for the duration of the therapy.) Kaplan-Meier survival estimates were plotted and Cox's proportional hazard ratios were calculated. No associations with any of the exposure variables were noted but the confidence intervals were very wide.

Results when mosquitos were used on more than one day

Episodes where mosquitos were given over 2 or 3 days were analysed separately. The same exclusion criteria were used as before. The mosquito number was taken as the total number used over the three days, and the prepatent and incubation periods were calculated from the first day when mosquitos were used. 231 episodes fulfilled the criteria, of which 110 did not receive treatment within the first five days of patent parasitaemia.

Mosquito number was inversely related to prepatent and incubation periods. Longer storage times led to longer prepatent and incubation periods, and these two effects were independent.

The pretreatment peak parasitaemia was weakly correlated with the number of infected mosquitos and the total number of mosquitos used, but these associations were reduced after adjusting for year of treatment. There were no associations between prepatent period, incubation period or storage time and pretreatment peak parasitaemia. The results using overall peak parasitaemia were similar, although the effects of adjusting for year were less marked.

No associations were found between any of the measures of dose and the peak temperature before treatment or overall, either in the crude analysis or after adjusting for confounders. There were no associations between measures of dose and the number of paroxysms over 103°F pretreatment. Using the overall number of paroxysms, after adjusting for time to treatment, weak negative correlations with prepatent and incubation period were found, but these were lost after adjusting for the other confounders.

Episodes of tertian fever overall were more common in those bitten by fewer mosquitos and fewer infected mosquitos and in those where the mosquitos were stored for longer than 20 days (13/18 of those stored ≥ 20 days compared with 38/90 stored < 20 days). After adjusting for confounders the associations with the number of mosquitos were lost but the association with storage time became more marked. There were no associations with prepatent or incubation period.

Episodes of tertian fever pretreatment were also more common when mosquitos were stored for more than 20 days (7/14 compared with 10/75). This persisted after adjusting for confounders. No associations were found between this outcome and any of the other measures of dose in either crude or multivariate analyses. Pure tertian fever pretreatment and overall was only found in three patients.

Spontaneous recovery was recorded for 13 patients and "very ill" for 7. There were no associations between either of these outcomes and any of the measures of dose in crude or multivariate analyses. Only two of the patients died.

Modifying treatment was given to 53 patients. Those bitten by more mosquitos or more infected mosquitos were less likely to receive modifying treatment. After adjusting for year

these relationships persisted, but using the number of infected mosquitos the association was of borderline significance. Modifying treatment was given less frequently after longer prepatent and incubation periods and was not associated with storage time (Table 3.3.9).

There were no associations between the measures of dose and the number of doses of modifying treatment. Nor were there any associations with the time elapsing before treatment which were independent of whether modifying treatment was given. In five patients the parasitaemia ended before full treatment was given. This outcome was not significantly associated with any of the measures of dose. In only two did the parasitaemia end before any treatment was given.

Results obtained by using both mosquito number and storage time to determine dose groups
Patients bitten on only one day, selected using the criteria given above, were divided into groups according to the estimated number of infected mosquitos used and the storage times. Those bitten by at least five mosquitos which had been stored for less than 10 days were regarded as the high dose group (n = 91). Those bitten by less than five mosquitos which had been stored for at least 10 days were regarded as the low dose group (n = 49). The rest of the patients were excluded from this analysis.

Patients in the high dose group had much shorter prepatent and incubation periods (Table 3.3.10). For continuous outcome variables the means (or geometric means) were compared between the two groups. For binary outcome variables results were assessed using two-by-two tables. Few differences were found in the distributions of the outcome variables between the two groups. The pretreatment peak temperature was slightly higher in the high dose group. The proportion of days pretreatment with peaks over 103°F was also higher in the high dose group, but there was no difference in the crude number of peaks before treatment.

Trophozoite-induced malaria

Information was available for 241 trophozoite inoculations of which 217 had further information available from the second set of books or notes. 230 (95.4%) of the inoculations led to parasitaemia and fever. (One additional patient had low grade parasitaemia alone and died of bronchopneumonia within 4 days. He was not included in the analysis.)

For the analysis the same exclusion criteria were applied as previously: patients were excluded

if they had had previous malaria therapy with the same type of malaria (including unsuccessful inoculations), if they were known to have had natural malaria in the past, if the prepatent period was equal to or greater than 30 days, or if inoculations were given on more than one day. This left 166 patients. For all of the analyses except the comparison of dose with prepatent and incubation periods, patients receiving treatment within the first five days were excluded (leaving 107 patients).

Dose, prepatent period and incubation period were all approximately log-normally distributed. The route of infection was only known for 47 patients, of whom 44 received intramuscular injections and 3 intravenous. The distribution of incubation and prepatent period curves overall and for those receiving known intramuscular injection were very similar (Fig 3.3.14), suggesting that the vast majority of the injections were intramuscular. After excluding patients who were treated in the first five days, none were known to have had intravenous inoculations. The mean estimated number of parasites inoculated was approximately 3×10^7 and the median 1.7×10^7, with a range of 2.5×10^7 to 48×10^7. Almost all of the patients were inoculated between 1936 and 1950. Their mean age was 45 (range 7 to 69), and 65% were male.

The relationship between dose and prepatent and incubation periods

Log prepatent period was weakly, negatively correlated with log dose:

r = -0.24 (95% CI -0.38 to -0.08)

b = - 0.063 (-0.10 to -0.022)

a = 1.41, F = 9.01

A similar relationship was seen for incubation period, and neither was altered by adjusting for sex, age or year (Table 3.3.11).

Results relating to parasitaemia

There were no associations between any of the measures of exposure and the peak parasitaemia reached before treatment or overall (Tables 3.3.12-13). The peak parasitaemia increased slightly with age but was not associated with sex or year. No associations with the exposure variables were found after adjusting for the possible confounders.

Results relating to temperature

The peak temperature overall, but not the peak temperature occurring before treatment, decreased with increasing dose (Table 3.3.12). This persisted after adjusting for the possible confounders. No associations were found between peak temperature and either prepatent period

or incubation period, before or after adjusting for confounders (Table 3.3.13). The peak temperature before treatment and overall decreased with age, but did not vary with sex or the year of treatment.

The number of paroxysms over 103°F before treatment and overall were strongly related to the time between patent parasitaemia and treatment. The overall number of paroxysms decreased with age. There were no associations between any of the exposure measures and the number of paroxysms before treatment or overall, with or without adjusting for the time to treatment or other possible confounders (Tables 3.3.12-13).

No associations were found between dose and the occurrence of episodes of tertian fever or predominantly tertian fever overall or before treatment (Table 3.3.14). Episodes of tertian fever both before treatment and overall were more frequent following longer prepatent and incubation periods (Table 3.3.15). The trend overall was not affected by adjusting for the confounders, but was seen predominantly before 1940. The trend before treatment was more marked after adjusting for the time to treatment, and was seen mainly among the women.

Predominantly tertian fever before treatment and overall also occurred more frequently following longer prepatent and incubation periods. After adjusting for year the association between prepatent period and predominantly tertian fever overall remained of borderline significance, but the other associations were lost. Predominantly tertian fever was recorded more commonly before 1940. There were no associations between age or sex and any of the measures of tertian fever.

Results based on descriptions

Only three deaths during patent parasitaemia were recorded. Seven patients were recorded as being "very ill". There were no associations between being very ill and any of the measures of exposure, before or after adjusting for possible confounders.

Thirteen patients were reported to have made a spontaneous recovery. This was more likely to occur following smaller doses, but was not associated with prepatent or incubation period (Tables 3.3.14-15). Spontaneous recovery was more likely at younger ages and in earlier years; the results were not changed by adjusting for possible confounders.

Results relating to treatment

Full treatment was given to all but 3 patients. Modifying treatment was given to 47 (43.9%), at least two doses to 12 and at least three to 7. More modifying treatment was given in the later years and slightly more at older ages. The parasitaemia ended before full treatment was given in 13 cases and before any treatment in 6. No associations were found between these outcomes and any of the measures of exposure (Tables 3.3.14-15).

The time between patent parasitaemia and the first treatment was not associated with any of the exposure variables. The time between patent parasitaemia and starting full treatment decreased with increasing dose and increased with increasing incubation periods. It increased slightly with increasing prepatent period but this was less marked. These associations persisted after adjusting for possible confounders, including whether modifying treatment was given.

Whether modifying treatment was given, the number of doses of modifying treatment, and the time between patent parasitaemia and the first modifying treatment were not associated with prepatent or incubation period. Modifying treatment was not associated with dose in the crude analysis, but, after adjusting for year, modifying treatment was found to be given slightly less often following higher doses. The timing of modifying treatment was not associated with dose (Tables 3.3.14-15).

Sporozoite-induced malaria

138 patients received sporozoite inoculations of whom 111 developed fever and parasitaemia. One further patient, who had had previous malaria therapy with *P vivax* had parasitaemia alone. After excluding the 24 patients where the treatment of the mosquitos or sporozoites was unusual (see methods) and patients who had had previous malaria therapy with vivax or natural malaria, the attack rate was 92.7% (89/96). For the analysis the same exclusion criteria were applied as previously: patients were excluded if they had had previous malaria therapy with vivax malaria, previous natural malaria, prepatent periods over 29 days, or inoculations on more than one day. This left only 83 successful inoculations of which the batch of mosquitos was known in 73, and information from the second set of books was available for 79. An estimate of the number of sporozoites was available for 60 of the successful inoculations. As before, for all of the analyses except the comparison of dose with prepatent and incubation periods, patients receiving treatment within the first five days of patent parasitaemia were excluded, leaving 57 patients among whom an estimate of sporozoite numbers was available

for 42.

The estimated number of sporozoites was approximately log normally distributed with a median of about 40,000, minimum of 800 and maximum of 440,000. One value of 8,400,000 was excluded in the calculations involving number because the sporozoites came from oocysts (see methods). The prepatent and incubation periods approximated to normal distributions with means of 12.7 and 10.8 respectively. Two thirds of the patients were male and half were over 45. The date of the inoculation ranged from 1927 to 1959 with the majority being carried out in the two years 1937 and 1945.

The relationship between dose, storage time and prepatent and incubation periods

The prepatent period was inversely related to the number of sporozoites and was longer with longer storage times (Figure 3.3.15). The incubation period showed little relationship with the number of sporozoites but was also longer with longer storage times (Figure 3.3.16).

Results relating to measures of severity

The relationships between sporozoite numbers and prepatent period with various outcome measures are shown in Tables 3.3.16-19. No strong associations between the exposure and outcome variables were found. The occurrence of tertian fever before treatment increased with longer prepatent periods, but this association was reduced after adjusting for confounding by age. The time to first drug treatment increased with prepatent period, but no associations with other measures of treatment were found. The numbers are small in many of the groups and no results were possible for some of the outcome variables used in the other types of malaria therapy (see tables).

DISCUSSION

For all three types of induced malaria the inverse relationship between dose and prepatent and incubation periods was confirmed. With some notable exceptions, there was little correlation between the estimates of dose and any of the measures of severity (Table 3.3.20). Some of the problems relating to the measurements of dose were discussed in the review of the literature. In mosquito-induced infections the number of mosquitos used is likely to correlate only poorly with the number of viable sporozoites injected: not all the mosquitos in a batch are always infected; the storage time appears to affect the viability of the sporozoites; and the number of

sporozoites transmitted by individual mosquitos is highly variable. While it was possible to adjust for the probable proportion of infected mosquitos and for the storage time, the number of sporozoites transmitted remains unknown.

For sporozoite-induced malaria the handling of the sporozoites may have affected their viability. The attack rate was similar in non-immunes bitten by mosquitos and those given direct sporozoite injection. However, since the number of sporozoites given directly was very much larger than that likely to have come from bites, a large fall off in viability could occur without affecting the attack rate. The geometric mean prepatent period for sporozoite-induced infection was less than a day shorter than that for mosquito-induced infection (12.02 days, 95% CI 11.48-12.60, compared to 12.88, 95% CI 12.66-13.11, Figure 3.3.17). This suggests a considerable loss in numbers of viable sporozoites. It is likely that the proportion of sporozoites remaining viable varied from inoculation to inoculation. It has been suggested that the counts were not very accurate, perhaps because sporozoites tend to adhere to glass vessels (Roy 1938). The storage of the mosquitos before the glands were dissected could also affect the outcome but could be adjusted for.

For trophozoite-induced infections it was assumed that the blood was taken from the donor patient at the same time as the parasite count was done. It is known that when compatible blood is used the incubation period is much shorter than when incompatible blood is used (Polayes & Derby 1934), presumably indicating that a higher proportion of the injected trophozoites survive. However in this data set no blood groups were available. For both sporozoite- and trophozoite-induced infections all the injections were assumed to be by the same route (intravenous and intramuscular respectively); it was known from the larger data set that the majority but not all of the infections were by these routes.

Since the measures of dose correlated as expected with the prepatent and incubation periods they must also correlate to some extent with the "true" dose. It is possible that the prepatent and incubation period provide better measures of the true dose because they can be expected to vary with some of the factors which affect the true dose but are unrecorded, such as the number of sporozoites in mosquito-induced infection, the viability of the sporozoites or the blood compatibility. Another advantage of using the prepatent or incubation period rather than the number of mosquitos in mosquito-induced malaria is that it was possible to split the exposure variable into more groups reflecting a wider range of dosages: in the selected data set of those bitten on one day, around 80% of patients were bitten by between 5 and 15

mosquitos, or between 3 and 10 infected mosquitos. The disadvantage is that variations in patient immunity and susceptibility could confound any relationship between dose and severity which uses the prepatent or incubation period as measures of dose since they would be expected to influence both the prepatent or incubation period and the outcome. In this data set none of the patients were immune (patients who were known to have had previous natural malaria or previous malaria therapy with the same species were excluded). This lack of immunity is reflected in attack rates of over 90%. Variations in susceptibility due to age and sex were controlled for in the analysis. All the patients were white, and most were English.

"Low" and "high" dose groups were defined on the basis of both numbers and storage time as a response to the marked effect of both of these variables on prepatent and incubation period, and as an attempt to create two distinct groups for comparison. However, as the mosquito numbers used for most patients came from a small range, the groups could not be very distinct. Thus 34/48 in the low dose group were bitten by at least 3 mosquitos, and 45/90 in the high dose group were bitten by fewer than 7. The failure to find associations between these groups and most of the outcome measures may reflect this concentration of the distribution. But, since these dose groups were highly predictive of prepatent and incubation period, it does also suggest that the correlations found with outcome measures when using prepatent and incubation period in the other parts of the analysis could be due to confounding by susceptibility.

In this data set the recorded prepatent period is longer than the incubation period, which is the reverse of the usual pattern. The restriction of examinations to thin films will have delayed the first recording of patent parasitaemia, and the use of regular temperature charts before the first clinical signs of fever will have led to earlier recordings of the fever. The prepatent and incubation periods were highly correlated, with only rarely more than three days between them for any individual patient and the results obtained using them as measures of dose were very similar.

For both mosquito-induced and trophozoite-induced infections, longer prepatent and incubation periods were associated with increased proportions of patients showing tertian fever. (For sporozoite-induced infections the numbers were very small.) No associations between the more direct measures of dose and tertian fever were found in this study.

Apart from the occurrence of tertian fever, the only outcome measures which were strongly

correlated with exposure variables were those which relied on a clinical assessment of the patient: spontaneous recovery and whether modifying treatment was given. In mosquito-induced malaria where mosquitos were used on only one day, the proportion of patients undergoing spontaneous recovery approximately doubled with every two day increase in prepatent period (Table 3.3.6). Modifying treatment was more likely to be given to patients bitten by more mosquitos and with longer prepatent or incubation periods (Table 3.3.8). However, the results found when mosquitos were given on two or three days contradicted the results relating modifying treatment to mosquito number: a surprising negative correlation between mosquito number and giving modifiying treatment was found, although the results with prepatent and incubation period confirmed the previous findings.

According to the published reports (Nicol 1927, Shute 1952), spontaneous recovery was used to describe recovery from fever, often despite persistent parasitaemia. What it meant in practice is difficult to assess. Half (22/43) of the patients said to have had a spontaneous recovery in mosquito-induced malaria received modifying treatment (compared to two thirds, 140/215, of the others). And two patients who were not recorded as having had a spontaneous recovery stopped having patent parasitaemia before any treatment was given (Table 3.3.7).

The published reports suggest that modifying treatment was given in response to severe symptoms or high parasitaemias (Nicol 1927, Shute 1952). If this were always the case it would make an excellent overall marker for severe disease but it was often given so early that there must be some doubt. The analyses excluded patients treated within the first five days of patent parasitaemia; within the resulting subset of patients the use of modifying treatment may have been more closely related to severity of disease. For occasional patients the reason for giving the modifying treatment was stated. The reason usually related to symptoms but was occasionally administrative (such as for Christmas). Modifying treatment was also given when the patient's overall state of health was poor, which depended on the neurosyphilis as well as the malaria. Modifying treatment was given much more frequently and earlier in more recent years. This was adjusted for in the analysis, but suggests that the criteria for giving treatment changed.

A few other correlations between exposure and outcome variables were found which approached or reached statistical significance at the 5% level. Some were in the direction to support a dose-severity relationship, others not. They are all mentioned in the results sections. Their true significance is dubious. Any analysis which makes as many comparisons as this can

be expected to produce some "significant" results by chance and none of these results were consistent between related outcome variables or related exposure variables. Surprising correlations were found between prepatent and incubation periods and the outcome "very ill" (Table 3.3.6), but it was difficult to tell when entering the data whether the comments referred to the underlying state of the patient from their neurosyphilis or to the malarial illness.

The lack of association of the exposure variables with the more objective outcome measures - parasitaemia, peak fever, the number of peaks over 103°F, and death - is striking. So how accurate are these outcomes?

As mentioned in the methods, there is a discrepancy in the two published accounts regarding the eye-piece used on the microscope when parasite counts were done, suggesting a change in practice some time between 1927 and 1952 (Nicol 1927, Shute 1952). When this change occurred is unknown, but, if it represents a real change and not a typographical error, it means that the recorded number of parasites per high power field will have had a different interpretation in terms of percentage parasitaemia at one time from another. For the analysis a consistent interpretation has been assumed. Controlling for the year of the malaria therapy will not have effectively adjusted for the change, as year was treated as a linear variable or in a few groups, and the change occurred once at an unknown time. Even without these problems the parasite counts recorded may not accurately represent the true peak counts as they were done only once a day. Neither the actual timing of the blood sample, nor the timing in relation to symptoms were recorded.

The temperature was recorded to the nearest 0.2°F. Nicol (1927) noted that attempts were made to lower the fever when it reached 105°F so some potentially higher fevers would have been lowered. There is an excess of peak fevers of 104.8°F (Fig 3.3.18) suggesting that peaks may have been under-recorded to avoid cold sponging. The measurement of the number of peaks over 103°F avoids these problems but is obviously very dependent on the time elapsing before treatment. In the analysis this was controlled for in several ways; in none of the data sets was the number of peaks over 103°F before treatment or overall, associated with the direct measures of dose, nor with the other exposure variables after allowing for confounding.

Death, while an objective endpoint in itself, was not necessarily due to the malaria. Indeed a few patients, who were not included in the analysis, died during the incubation period, indicating the severity of the neurosyphilis and its associated complications in some cases.

Given the inaccuracies of measurement of both dose and outcome which lead to non-differential misclassification and therefore underestimation of any associations, it is difficult to reach conclusions of no effect from the data. However, the finding of the expected associations between dose and prepatent and incubation periods is evidence that the measures of dose reflect the true dose to some degree. Only the relationships between prepatent and incubation period and the occurrence of tertian fever and the use of modifying treatment were found in more than one of the data sets, and no consistent relationships were found with the more direct measures of dose.

PLASMODIUM OVALE

BACKGROUND

Although other types of malaria were used at the Horton, the experience with other individual strains transmitted by a single route was very limited. Considering only those patients with successful inoculations who had not had previous malaria therapy with the same strain and who were not treated within the first 5 days, only one other strain was given to sufficient patients to make analysis worthwhile. (Other strains were given to a maximum of 50 patients.) This was a strain of *P ovale* first isolated from a patient who had come from the Gold Coast in 1939, referred to as West African strain V. It was maintained mostly by person-to-person transmission until 1943 when it became mixed with vivax (presumably due to an accidental infection or a relapse).

METHODS

Data was only entered on patients who had had no previous malaria therapy with *P ovale*, received blood inoculation on one day only, had had no prophylactic treatment, and were not treated within the first five days of patent parasitaemia. The calculation of dose and the methods of analysis were the same as for the Madagascar strain of vivax malaria.

RESULTS

81 patients fulfilled the above criteria. One was excluded as he had had previous natural malaria. The route of injection was not recorded on any of the patients. Dose, prepatent period, incubation period, and peak parasitaemia approximated to log normal distributions, the other continuous variables approximated to normal distributions. All analyses were repeated adjusting for possible confounders: age, sex, year of treatment, and time of treatment.

No relationship was demonstrated between dose and either prepatent or incubation period.

In contrast to vivax malaria, the majority of patients treated with this strain appeared to be

recovering before any treatment was given. 57/80 had spontaneous recovery recorded in their notes, and 45/77 had parasitaemias of ≤ 1 per 100 high power fields when treatment was begun. Modifying treatment was only given to one patient, and there were no differences in the outcome measures (peak parasitaemia, peak fever, type of fever or number of peaks over 103°F) whether they were recorded pretreatment or overall.

No associations were found between the peak parasitaemia and the calculated dose, the prepatent period or the incubation period. The peak temperature was not associated with the dose or the incubation period, but there was a weak inverse relationship between peak temperature and log prepatent period: r = -0.22 (95%CI -0.42 to 0.00), b = -1.55 (95% CI -3.08 to -0.022). The regression coefficient was closer to zero after adjusting for time lapsing before treatment, but the association persisted in those who had a spontaneous recovery.

There was an inverse correlation between both prepatent period and incubation period and the number of peaks over 103°F. The correlations were less strong but were still seen after adjusting for time to treatment. In both cases the relationships were marked among those who had made a spontaneous recovery (Table 3.3.21). Using log prepatent period the following relationships were found: overall, r = -0.34 (95% CI -0.52 to -0.13), b = -9.50 (95% CI -15.23 to -3.68); in those with spontaneous recovery, r = -0.44 (95% CI -0.63 to -0.20), b = -12.82 (95% CI -19.80 to -5.83). No relationships were found between the number of peaks over 103°F and the dose.

Tertian fever occurred in over half of the patients, and predominantly tertian fever in a quarter. No relationships were found between the dose or the prepatent or incubation period and the occurrence of episodes of tertian fever, or predominantly tertian fever.

Neither the dose nor the prepatent or incubation period predicted whether spontaneous recovery was recorded. Full treatment was given to all but 8 patients. It appeared that it was given early either because the clinical infection was nearly over anyway or because the infection was severe. The time to treatment overall was therefore not a useful measure of severity. For those who were recorded as making a spontaneous recovery, the time to treatment or to the end of the patent parasitaemia if earlier was calculated as a measure of the duration of infection. The duration was not related to dose, but was inversely correlated with both prepatent period and incubation period (Table 3.3.22). Using log prepatent period and log duration: r = -0.38 (95% CI -0.58 to -0.13), b = -0.58 (95% CI -0.95 to -0.20).

DISCUSSION

In this data set no associations were found between dose and the prepatent period or incubation period or any of the outcome variables. The lack of association with prepatent and incubation period is surprising in light of the results with vivax malaria. I have found no information relating dose and outcome in induced ovale malaria in the published literature. As with vivax malaria, it was assumed in calculating the dose that the parasite count recorded for the donor on the day blood was taken was the same as that in the donated blood, although the two procedures may not have occurred at the same time. The date of the change in microscope eye-pieces is unknown, but it would be unlucky for it to have occurred in the four year period covered by this data. These problems and the lack of information on blood groups (meaning that some inoculations are compatible and others incompatible) may provide the explanation for this lack of association.

Similar relationships with the outcome measures were found using prepatent and incubation periods. The incubation periods were missing in some cases, usually because an early fever was attributed to blood inoculation. The prepatent periods are probably more reliable. No relationship was found between prepatent period and spontaneous recovery. The recording of spontaneous recovery in the notes is taken to refer to the fever, but from the parasitaemia records it appeared that almost all patients were beginning to recover before treatment was started, so it may not distinguish mild infections as well as for vivax.

Tertian fever before treatment was commoner than in vivax malaria, and in contrast to vivax malaria, it was not associated with prepatent period.

Because the majority of infections recovered spontaneously, both the number of peaks over 103°F and the duration of infection were useful measures of severity. Both were inversely associated with prepatent period.

Overall, in this data set, there is some evidence of a relationship between prepatent period and severity, but none of a relationship between dose and prepatent period or dose and severity. The lack of association with dose may be due to the problems in measuring dose in un-crossmatched patients, but the associations found with prepatent period may be due to confounding by susceptibility.

PLASMODIUM FALCIPARUM: AMERICAN DATA

MATERIALS AND METHODS

For falciparum malaria few patients were treated with any one strain at Horton, and most were treated early. A small malaria therapy data set was available from the USA, from neurosyphilis patients treated in mental hospitals in South Carolina and Georgia in the 1940s and 50s. No information was available on the handling or storage of the mosquitos. The practice of the South Carolina Hospital was to "allow up to 20 paroxysms if we think the patient can withstand this amount of treatment. As many as 25 or 30 paroxysms are allowed for Negroes." (Mayne & Young 1941).

The records of 108 patients were available. As far as was known, none of the patients had had previous malaria, although a few had had failed attempts to induce malaria using other strains. Three different strains of falciparum malaria were used: El Limon (EL) McLendon (ML) and Santee Cooper (SC). Personal details were usually lacking: all 41 of the patients for whom details were recorded were male and Black.

The data was analysed to look for an association between dose and severity of outcome. Almost all of the infections in the data set were induced using mosquito bites: the few where direct inoculation of sporozoites was used were excluded from the analysis.

The measures of dose available were the number of mosquitos used and the sum of the number of pluses, based on a scoring system from the number of sporozoites seen in the gland of dissected mosquitos. Summing the pluses in this way makes the assumption that being bitten by one mosquito scoring 4+ (equivalent to >1000 sporozoites in the glands) is equivalent to being bitten by four mosquitos scoring 1+ each (equivalent to 1 - 10 sporozoites in the glands). Since there is no basis for this assumption (the number of sporozoites ejected correlates poorly with the gland load, as discussed above), since the number of pluses was not recorded on all the patients, and since, in practice, the number of pluses was highly correlated with the number of mosquitos, this measure was not used. The prepatent period was also investigated as a possible indirect measure of infective dose, as before.

Several measures of severity were available. The most general was whether the patient received

any treatment during the acute phases of the infection (taken as the first twenty days of patent parasitaemia). Daily counts and any fevers over 101°F during this twenty day period were recorded, together with the total duration of the parasitaemia and whether any later treatment was given. Patients were treated with cool sponging and cold drinks during febrile episodes (Mayne & Young 1941).

RESULTS

Eight patients were excluded because they had received direct inoculations of sporozoites instead of or as well as mosquito bites. Fifty patients received EL, 39 ML and 10 SC. There were differences between the strains in the number of mosquitos used, the prepatent period and the outcome, so any analyses have to take account of this (see Table 3.3.23)

The number of mosquitos used, the prepatent periods, the parasite counts and the duration of parasitaemia all approximated more closely to lognormal distributions than normal distributions, so log values were used for the analyses. There was a weak inverse correlation between the number of mosquitos used and the prepatent period but this was lost after adjusting for strain.

Four different types of mosquito were used: *An quadrimaculatus*, *An albimanus*, *An crucians* and *An freeborni*. The first two were used for the vast majority of the infections, mainly *An albimanus* for strain EL and *An quadrimaculatus* for strain ML. Since it is possible that different mosquito species transmit different numbers of sporozoites, when the strains were considered separately using mosquito number as the measure of dose, analyses were repeated using just the predominant mosquito species (Table 3.3.24). This had little effect on the results.

No information describing the criteria for giving treatment was available. It is possible that, as in the Horton data, treatment given very early was unlikely to be a response to the severity of the malaria. Comparing the mean pretreatment peak parasitaemias in those treated on different days, it appeared that those treated after about day five had peak parasitaemias as high as the rest whereas those treated earlier had lower peaks. Those treated within the first five days were therefore excluded from the analysis, leaving 82 patients.

There was no relationship between the number of mosquitos used and whether early treatment

(ie treatment between 6 and 20 days) was given. Of those bitten by fewer than 15 mosquitos, 19/35 (54.3%) received early treatment, compared with 26/46 (56.5%) of those bitten by 15 or more. The relative risk was unchanged after stratification by strain. There were no associations found between number of mosquitos and early treatment when strains EL and ML were analysed separately.

To assess whether the prepatent period was related to the need for early treatment the strains were only considered separately because of the different ranges of prepatent period. For EL, 19/26 (73.1%) of those with prepatent periods of 10 days or less received early treatment, compared with 19/24 (79.2%) of those with longer prepatent periods.

For ML, 11/15 (73.3%) of those with prepatent periods of 12 days or less received early treatment, compared with only 7/19 (36.8%) of those with longer prepatent periods. The relative risk of early treatment following a short prepatent period was 1.99 (95% CI 1.03 - 3.86). There was no association using ML or EL between prepatent period and the time of the early treatment.

For strains EL and ML the two measures of dose - mosquito number and prepatent period - were compared with pretreatment peak parasitaemia, pretreatment peak fever, and the proportion of days occurring pretreatment, or in the first 20 days, on which fevers of over 103°F and over 101°F occurred. No associations were found with any of these measures using either strain, either in the crude analysis or after controlling for early treatment and/or the timing of the treatment.

The remaining analyses considered only those who had received no early treatment. They were carried out for the three strains together, controlling for strain by multiple regression, and for strains EL and ML separately.

No associations were found between the number of mosquitos employed and the peak parasitaemia, the geometric mean parasitaemia over the first twenty days, the peak fever reached, the number of days of fever over 101°F or over 103°F in the first twenty days, or the total duration of parasitaemia (in those who received either no treatment or treatment commencing at least 30 days after the last parasitaemia).

No associations were found between the prepatent period and any of the measures of severity

except the number of days of fever over 103°F, and this was lost after controlling for strain.

DISCUSSION

The data set is small but provides rare information on patients with untreated falciparum malaria bitten by a known number of mosquitos.

No associations were found between the number of mosquitos employed and any of the measures of severity. However there were several problems with the data, in particular the lack of personal information about the patients and the lack of information about the storage time of the mosquitos, which may affect the infectivity of the sporozoites.

As the transmission of sporozoites by mosquitos is very variable, the prepatent period was considered as an alternative measure of dose. For the ML strain, patients with shorter prepatent periods were more likely to receive treatment within the first 20 days. This was not found for the EL strain, and no other evidence of an association between dose and severity was found.

Table 3.3.1. *P vivax*. The relationship between the number of mosquitos used and the prepatent period

			Prepatent period	
		n	Geometric mean	95% CI
Number of infected mosquitos	< 5	151	13.55	13.16-13.96
	5 - 9	270	13.06	12.76-13.37
	10 - 19	86	12.59	11.44-13.15
	≥ 20	31	10.76	10.15-11.42
Total number of mosquitos used	< 5	49	13.77	12.97-14.63
	5 - 9	251	13.18	12.88-13.49
	10 - 19	189	13.00	12.64-13.37
	≥ 20	49	11.25	10.65-11.87

Table 3.3.2. *P vivax*. The relationship between the number of mosquitos used and the incubation period

			Incubation period	
		n	Geometric mean	95% CI
Number of infected mosquitos	< 5	151	12.19	11.81-12.58
	5 - 9	269	11.48	11.20-11.77
	10 - 19	86	11.17	10.69-11.67
	≥ 20	31	9.64	8.93-10.40
Total number of mosquitos used	< 5	49	12.30	11.59-13.06
	5 - 9	251	11.75	11.47-12.04
	10 - 19	188	11.38	11.03-11.73
	≥ 20	49	10.05	9.48-10.65

Table 3.3.3. *P vivax*. The relationship between the estimated number of infected mosquitos used and various measures of severity (unadjusted for any confounders).

		Number of infected mosquitos		
		< 5	5 - 9	≥ 10
Peak parasitaemia (per 100 high power fields)	n	70	167	50
	Geometric mean	57.02	60.81	57.02
	95% CI	48.37-67.21	55.42-66.73	47.90-67.87
Pretreatment peak parasitaemia (per 100 high power fields)	n	70	167	50
	Geometric mean	47.42	53.95	50.82
	95% CI	39.82-56.48	48.36-60.18	40.41-63.90
Peak temperature (°F)	n	61	153	41
	Mean	105.1	105.2	105.2
	95% CI	105.0-105.3	105.1-105.3	105.0-105.3
Pretreatment peak temperature (°F)	n	33	98	27
	Mean	105.0	105.1	105.1
	95% CI	104.9-105.2	105.0-105.2	104.8-105.3
No. of peaks over 103°F	n	61	153	41
	Mean	10.31	10.52	9.49
	95% CI	9.65-10.97	10.07-10.96	8.51-10.46
No. of peaks over 103°F pretreatment	n	33	95	27
	Mean	9.52	8.76	8.19
	95% CI	8.34-10.69	8.10-9.42	6.78-9.59
No. of peaks over 103°F divided by time to treatment	n	61	153	41
	Mean	1.08	1.14	1.04
	95% CI	0.96-1.20	1.07-1.21	0.92-1.15
No. of peaks over 103°F pretreatment divided by time to treatment	n	33	95	27
	Mean	0.70	0.81	0.80
	95% CI	0.62-0.79	0.76-0.86	0.70-0.90
Days between patent parasitaemia and treatment	n	69	166	49
	Mean	10.90	10.11	9.71
	95% CI	9.77-12.03	9.47-10.75	8.68-10.75

Table 3.3.4. *P vivax.* The relationship between prepatent period and various measures of severity in mosquito-induced infections (unadjusted for any confounders).

		Prepatent period (days)				
		< 12	12-13	14-15	16-17	≥ 18
Peak parasitaemia (per 100 high power fields)	n	56	92	74	43	23
	Geometric mean	55.6	56.8	64.3	63.1	53.2
	95% CI	46.4-66.6	49.5-65.1	56.3-73.3	52.3-76.1	40.8-69.4
Pretreatment peak parasitaemia (per 100 high power fields)	n	56	92	74	43	23
	Geometric mean	45.6	50.2	54.8	60.4	46.8
	95% CI	36.2-57.5	43.2-58.5	46.3-65.0	50.2-72.7	35.5-61.6
Peak temperature (°F)	n	50	79	68	38	21
	Mean	105.2	105.2	105.1	105.0	105.2
	95% CI	105.0-105.3	105.1-105.3	105.0-105.3	104.9-105.2	104.9-105.5
Pretreatment peak temperature (°F)	n	17	49	46	29	18
	Mean	105.0	105.2	105.0	105.0	105.2
	95% CI	104.7-105.2	105.0-105.3	104.9-105.2	104.8-105.2	104.9-105.5
No. of peaks over 103°F	n	50	79	68	38	21
	Mean	10.14	10.46	10.78	9.84	9.38
	95% CI	9.29-10.99	9.88-11.03	10.08-11.48	9.06-10.62	8.00-10.76
No. of peaks over 103°F pretreatment	n	17	47	45	29	18
	Mean	9.24	8.81	9.02	8.55	8.17
	95% CI	7.43-11.04	7.80-9.82	8.01-10.03	7.36-9.74	6.48-9.86
No. of peaks over 103°F divided by time to treatment	n	50	79	68	38	21
	Mean	1.09	1.18	1.14	1.04	0.93
	95% CI	0.98-1.20	1.07-1.29	1.04-1.24	0.89-1.18	0.72-1.14
No. of peaks over 103°F pretreatment divided by time to treatment	n	17	47	45	29	18
	Mean	0.77	0.81	0.82	0.77	0.71
	95% CI	0.65-0.89	0.73-0.87	0.75-0.88	0.67-0.88	0.57-0.84
Days between patent parasitaemia and treatment	n	56	91	74	43	21
	Mean	9.82	9.85	10.14	11.14	11.43
	95% CI	8.89-10.76	8.96-10.73	9.26-11.01	9.58-12.70	9.04-13.81

Table 3.3.5. *P vivax*. The relationship between the estimated number of infected mosquitos used and various measures of severity (unadjusted for any confounders).

		Number of infected mosquitos			χ^2 linear trend	p
		< 5	5 - 9	≥ 10		
Episodes of tertian fever	n	36/68	87/166	30/47		
	%	52.9	52.4	63.8		
Episodes of tertian fever pretreatment	n	11/54	22/137	7/33		
	%	20.4	16.1	21.2		
Predominantly tertian fever	n	2/68	8/166	5/47		
	%	2.9	4.8	10.6	2.3	0.1
Predominantly tertian fever pretreatment	n	1/54	8/137	4/33		
	%	1.9	5.8	12.1	3.0	0.09
Spontaneous recovery	n	8/61	24/154	9/41		
	%	13.1	15.6	22.0	1.0	0.3
"Very ill"	n	6/61	10/154	3/41		
	%	9.8	6.5	7.3		
Death during parasitaemia	n	1/70	4/167	1/50		
	%	1.4	2.4	2.0		
End parasitaemia before full treatment	n	4/69	17/166	5/49		
	%	5.8	10.2	10.2		
End parasitaemia before any treatment	n	2/70	5/166	2/50		
	%	2.9	3.0	4.0		
Modifying treatment given	n	40/70	111/167	33/50		
	%	57.1	66.5	66.0		
At least 2 doses modifying treatment given	n	15/70	32/167	16/50		
	%	21.4	19.2	32.0		
At least 3 doses of modifying treatment given	n	6/70	6/167	7/50		
	%	8.6	3.6	14.0		
Full treatment given	n	68/69	164/167	48/50		
	%	98.6	98.2	96.0		

Table 3.3.6. *P vivax.* The relationship between prepatent period and various measures of severity in mosquito-induced infections (unadjusted for any confounders).

		Prepatent period					χ^2 linear trend	p
		< 12	12 - 13	14 - 15	16 - 17	≥ 18		
Episodes of tertian fever	n	34/55	45/88	38/73	23/43	15/23		
	%	61.8	51.1	52.1	53.5	65.2		
Episodes of tertian fever pretreatment	n	4/39	7/72	10/61	11/34	9/19		
	%	10.3	9.7	16.4	32.4	47.4	16.5	< 0.001
Predominantly tertian fever	n	1/55	4/88	3/73	2/43	5/23		
	%	1.8	4.5	4.1	4.7	21.7	6.3	0.01
Predominantly tertian fever pretreatment	n	0/39	2/72	3/61	4/34	3/19		
	%	0.0	2.8	4.9	11.8	21.1	11.5	< 0.001
Spontaneous recovery	n	2/50	7/78	11/68	12/39	11/22		
	%	4.0	9.0	16.2	30.8	50.0	28.5	< 0.001
"Very ill"	n	0/50	5/78	6/68	5/39	2/22		
	%	0.0	6.4	8.8	12.8	9.1	4.3	0.04
Death during parasitaemia	n	2/56	1/92	1/74	2/43	0/23		
	%	3.6	1.1	1.4	4.7	0.0		
End parasitaemia before full treatment	n	6/56	8/92	2/71	5/43	5/23		
	%	10.7	8.7	2.8	11.6	21.7		
End parasitaemia before any treatment	n	0/56	1/92	0/73	4/43	4/23		
	%	0.0	1.1	0.0	9.3	17.4	16.1	< 0.001
Modifying treatment given	n	43/56	63/92	48/74	23/43	9/23		
	%	76.8	68.5	64.9	53.5	39.1	11.9	< 0.001
At least 2 doses modifying treatment given	n	19/56	24/92	13/74	6/43	1/23		
	%	33.9	26.1	17.6	14.0	4.3	11.6	< 0.001
At least 3 doses of modifying treatment given	n	8/56	7/92	3/74	1/43	0/23		
	%	14.3	7.6	4.1	2.3	0.0	7.7	0.005
Full treatment given	n	55/56	91/92	72/73	42/43	21/23		
	%	98.2	98.9	98.6	97.7	91.3		

Table 3.3.7. *P vivax*. The relationship between parasitaemia, treatment and "spontaneous recovery" in mosquito-induced malaria.

		End parasitaemia before full treatment		
		No	Yes	All
"Spontaneous recovery"	No	197	15 (7.1%)	212
	Yes	35	8 (18.6%)	43
	All	232	23	255

		End parasitaemia before any treatment		
		No	Yes	All
"Spontaneous recovery"	No	211	3 (1.4%)	214
	Yes	37	6 (14.0%)	43
	All	248	9	257

Table 3.3.8. *P vivax.* Relationships between four measures of dose and the modifying treatment given in infections induced by mosquito bites on only one day.

	Outcome: modifying treatment given					
	Unadjusted			Adjusted for year of treatment		
	OR (95% CI)	LRS	*p*	OR (95% CI)	LRS	*p*
Number of infected mosquitos	1.29 (0.92-1.80)	2.3	0.1	1.57 (1.08-2.28)	5.8	0.02
Total number of mosquitos	1.41 (0.99-2.00)	3.8	0.05	1.50 (1.03-2.19)	4.7	0.03
Prepatent period	0.69 (0.56-0.85)	12.3	< 0.001	0.77 (0.62-0.96)	5.5	0.02
Incubation period	0.76 (0.63-0.93)	7.2	0.007	0.77 (0.62-0.94)	6.5	0.01
	Outcome: at least two doses of modifying treatment given					
	Unadjusted			Adjusted for year of treatment		
	OR (95% CI)	LRS	*p*	OR (95% CI)	LRS	*p*
Number of infected mosquitos	1.55 (1.08-2.22)	5.6	0.02	2.08 (1.34-3.24)	11.7	< 0.001
Total number of mosquitos	1.99 (1.35-2.93)	12.6	< 0.001	2.48 (1.55-3.96)	16.0	< 0.001
Prepatent period	0.63 (0.48-0.82)	12.8	< 0.001	0.74 (0.55-0.98)	4.6	0.03
Incubation period	0.73 (0.57-0.93)	7.1	0.008	0.74 (0.57-0.96)	5.6	0.02

The results were calculated using logistic regression with each exposure variable being categorised in five groups and treated as a linear variable. The groups used were, for number of infected mosquitos and total number of mosquitos: < 5, 5-9, 10-19, 20-39, ≥ 40; for prepatent period (in days): < 12, 12-13, 14-15, 16-17, ≥ 18; for incubation period (in days): < 10, 10-11, 12-13, 14-15, ≥ 16. Each line of the table refers to a separate model.

Table 3.3.9. *P vivax.* Relationships between four measures of dose and whether modifying treatment was given in infections induced by mosquito bites over 2 or 3 days.

	Outcome: modifying treatment given					
	Unadjusted			Adjusted for year of treatment		
	OR (95% CI)	LRS	*p*	OR (95% CI)	LRS	*p*
Number of infected mosquitos	0.49 (0.28-0.86)	6.7	0.01	0.55 (0.30-1.00)	4.1	0.04
Total number of mosquitos	0.54 (0.32-0.92)	5.5	0.02	0.49 (0.27-0.89)	5.9	0.02
Prepatent period	0.53 (0.32-0.88)	6.4	0.01	0.57 (0.33-0.97)	4.4	0.04
Incubation period	0.66 (0.41-1.06)	3.1	0.08	0.60 (0.36-0.99)	4.1	0.04

The results were calculated using logistic regression with each exposure variable being categorised in three groups and treated as a linear variable. The groups used were, for number of infected mosquitos: < 9, 9-13, ≥ 13; for total number of mosquitos: < 11, 11-15, ≥ 15; for prepatent period (in days): ≤ 12, 13-14, > 14; for incubation period (in days): ≤ 10, 11-12, > 12. Each line of the table refers to a separate model.

Table 3.3.10. *P vivax.* The relationship between dose groups (see text) and prepatent and incubation periods.

		Prepatent period (days)			Incubation period (days)		
		n	Geometric mean	95% CI	n	Geometric mean	95% CI
Dose group	Low	49	14.3	13.5-15.2	49	12.7	12.0-13.3
	High	90	11.9	11.5-12.3	90	10.5	10.1-10.9

Table 3.3.11. *P vivax*. The relationship between dose and prepatent and incubation periods for trophozoite-induced infections.

			Prepatent period	
		n	Geometric mean	95% CI
No. of parasites inoculated x 100000	≤ 80	42	10.23	9.13-11.47
	> 80 - ≤ 160	34	9.35	8.57-10.21
	> 160 - ≤ 320	38	8.55	7.60-9.62
	> 320 - ≤ 640	26	9.40	8.30-10.64
	> 640	14	8.07	6.82-9.56
			Incubation period	
		N	Geometric mean	95% CI
No. of parasites inoculated x 100000	≤ 80	42	8.39	7.32-9.63
	> 80 - ≤ 160	33	7.46	6.87-8.11
	> 160 - ≤ 320	37	7.19	6.32-8.19
	> 320 - ≤ 640	24	8.02	6.70-9.60
	> 640	13	6.19	5.25-7.31

Table 3.3.12. *P vivax*. The relationship between the estimated number of parasites inoculated and and various measures of severity in trophozoite-induced infections (unadjusted for any confounders).

		Estimated number of parasites x 100000				
		≤ 80	>80 - ≤160	>160 - ≤320	>320 - ≤640	> 640
Peak parasitaemia (per 100 high power fields)	n	26	21	25	19	10
	Geometric mean	56.8	69.7	55.5	71.3	64.6
	95% CI	41.6-77.4	46.9-103.5	38.9-79.1	45.6-111.5	22.1-188.7
Pretreatment peak parasitaemia (per 100 high power fields)	n	26	21	25	19	10
	Geometric mean	51.5	68.9	53.5	63.1	64.6
	95% CI	37.0-71.7	46.4-102.2	37.3-76.6	40.4-98.5	22.1-188.7
Peak temperature (°F)	n	24	20	23	18	6
	Mean	105.1	104.9	105.1	104.7	104.8
	95% CI	105.0-105.3	104.6-105.2	104.9-105.3	104.5-104.9	103.9-105.6
Pretreatment peak temperature (°F)	n	16	14	18	12	5
	Mean	105.0	104.8	105.0	104.7	104.8
	95% CI	104.8-105.2	104.4-105.3	104.7-105.3	104.5-105.0	103.7-105.8
No. of peaks over 103°F	n	24	20	23	18	6
	Mean	9.38	9.75	10.35	9.28	8.50
	95% CI	8.39-10.37	8.08-11.42	9.25-11.45	7.55-11.01	4.94-12.06
No. of peaks over 103°F pretreatment	n	16	14	18	12	5
	Mean	8.56	9.57	9.89	9.17	8.00
	95% CI	7.00-10.12	7.21-11.93	8.43-11.35	6.94-11.40	3.61-12.39
No. of peaks over 103°F divided by time to treatment	n	24	20	23	18	6
	Mean	0.93	0.90	0.93	0.99	0.92
	95% CI	0.79-1.07	0.71-1.09	0.74-1.12	0.74-1.12	0.78-1.21
No. of peaks over 103°F pretreatment divided by time to treatment	n	16	14	18	12	5
	Mean	0.77	0.75	0.73	0.83	0.86
	95% CI	0.64-0.90	0.57-0.93	0.61-0.85	0.66-1.01	0.19-1.53
Days between patent parasitaemia and treatment	n	26	20	25	19	10
	Mean	11.50	11.45	12.72	9.95	11.00
	95% CI	9.39-13.61	9.76-13.14	10.66-14.78	7.79-12.11	9.25-12.75

Table 3.3.13. *P vivax*. The relationship between prepatent period and various measures of severity in trophozoite-induced infections (unadjusted for any confounders).

		Prepatent period (days)				
		< 8	8-9	10-11	12-13	≥ 14
Peak parasitaemia (per 100 high power fields)	n	9	30	30	20	18
	Geometric mean	87.9	52.1	75.3	45.2	73.6
	95% CI	47.3-163.3	37.5-72.4	55.6-102.1	28.6-71.3	54.0-100.3
Pretreatment peak parasitaemia (per 100 high power fields)	n	9	30	30	20	18
	Geometric mean	83.6	52.1	69.2	40.7	69.8
	95% CI	44.2-158.0	37.5-72.4	49.9-96.0	23.8-69.6	50.2-97.2
Peak temperature (°F)	n	8	26	28	18	17
	Mean	105.0	105.0	104.9	105.1	105.0
	95% CI	104.3-105.6	104.7-105.3	104.7-105.1	104.9-105.4	104.7-105.2
Pretreatment peak temperature (°F)	n	7	21	20	11	9
	Mean	105.0	104.9	104.8	105.0	105.0
	95% CI	104.2-105.8	104.6-105.3	104.6-105.0	104.7-105.3	104.6-105.4
No. of peaks over 103°F	n	8	26	28	18	17
	Mean	9.50	9.85	9.86	10.06	8.35
	95% CI	6.74-12.26	8.51-11.19	8.77-10.95	8.60-11.52	7.06-9.64
No. of peaks over 103°F pretreatment	n	7	21	20	11	9
	Mean	9.43	9.48	8.95	9.18	8.11
	95% CI	6.15-12.71	7.87-11.08	7.34-10.56	6.78-11.58	6.25-9.97
No. of peaks over 103°F divided by time to treatment	n	8	26	28	18	17
	Mean	0.73	0.90	1.05	1.06	0.81
	95% CI	0.46-0.99	0.73-1.00	0.91-1.19	0.83-1.29	0.62-1.00
No. of peaks over 103°F pretreatment divided by time to treatment	n	7	21	20	11	9
	Mean	0.67	0.80	0.85	0.82	0.61
	95% CI	0.40-0.95	0.65-0.94	0.75-0.95	0.55-1.08	0.51-0.71
Days between patent parasitaemia and treatment	n	9	29	30	20	18
	Mean	13.44	12.59	10.03	10.55	11.67
	95% CI	8.75-18.14	10.99-14.18	8.48-11.50	8.69-12.41	9.22-14.11

Table 3.3.14. *P vivax*. The relationship between estimated number of parasites inoculated and various measures of severity in trophozoite-induced infections (unadjusted for any confounders).

		Estimated number of parasites x 100000					χ^2 linear trend	*p*
		≤ 80	>80 - ≤160	>160 - ≤320	>320 - ≤640	> 640		
Episodes of tertian fever	n	16/26	9/21	11/25	10/19	3/10		
	%	61.5	42.9	44.0	52.6	30.0	1.5	0.2
Episodes of tertian fever pretreatment	n	7/19	5/17	5/23	4/13	1/5		
	%	36.8	29.4	21.7	30.8	20.0		
Predominantly tertian fever	n	5/26	3/21	3/25	1/19	1/10		
	%	19.2	14.3	12.0	5.3	10.0	1.3	0.2
Predominantly tertian fever pretreatment	n	4/19	3/17	2/23	1/13	0/5		
	%	21.1	17.6	8.7	7.7	0.0	2.2	0.1
Spontaneous recovery	n	7/24	2/20	2/23	2/18	0/6		
	%	29.2	10.0	8.7	11.1	0.0	3.7	0.05
"Very ill"	n	2/24	1/20	1/23	1/18	2/6		
	%	8.3	5.0	4.3	5.6	33.3		
End parasitaemia before full treatment	n	4/26	2/20	1/25	0/19	3/10		
	%	15.4	10.0	4.0	0.0	30.0		
End parasitaemia before any treatment	n	1/26	2/21	1/25	0/19	2/10		
	%	3.8	9.5	4.0	0.0	20.0		
Modifying treatment given	n	13/26	9/21	10/25	10/19	1/10		
	%	50.0	42.9	40.0	52.6	10.0	1.5	0.2
At least 2 doses modifying treatment given	n	4/26	0/21	2/25	3/19	0/10		
	%	15.4	0.0	8.0	15.8	0.0		
At least 3 doses of modifying treatment given	n	2/26	0/21	2/25	2/19	0/10		
	%	7.7	0.0	8.0	10.5	0.0		

Table 3.3.15. *P vivax*. The relationship between prepatent period and various measures of severity in trophozoite-induced infections (unadjusted for any confounders).

		Prepatent period					χ^2 linear trend	p
		< 8	8 - 9	10 - 11	12 - 13	≥ 14		
Episodes of tertian fever	n	5/9	9/30	13/30	11/20	15/18		
	%	55.6	30.0	43.3	55.0	83.3	7.7	0.005
Episodes of tertian fever pretreatment	n	2/6	5/24	3/23	5/16	9/13		
	%	33.3	20.8	13.0	31.3	69.2	5.7	0.02
Predominantly tertian fever	n	1/9	3/30	1/30	3/20	6/18		
	%	11.1	10.0	3.3	15.0	33.3	4.0	0.05
Predominantly tertian fever pretreatment	n	1/6	2/24	1/23	2/16	5/13		
	%	16.7	8.3	4.3	12.5	38.5	3.3	0.07
Spontaneous recovery	n	0/8	7/26	2/28	2/18	3/17		
	%	0.0	26.9	7.1	11.1	17.6		
"Very ill"	n	1/8	3/26	1/28	0/18	2/17		
	%	12.5	11.5	3.6	0.0	11.8		
End parasitaemia before full treatment	n	0/9	6/29	1/30	2/20	4/18		
	%	0.0	20.7	3.3	10.0	22.2		
End parasitaemia before any treatment	n	0/9	4/30	0/30	1/20	1/18		
	%	0.0	13.3	0.0	5.0	5.6		
Modifying treatment given	n	3/9	12/30	13/30	10/20	9/18		
	%	33.3	40.0	43.3	50.0	50.0	0.9	0.3
At least 2 doses modifying treatment given	n	2/9	0/30	4/30	2/20	4/18		
	%	22.2	0.0	13.3	10.0	22.2		
At least 3 doses of modifying treatment given	n	1/9	0/30	3/30	2/20	1/18		
	%	11.1	0.0	10.0	10.0	5.6		

Table 3.3.16. *P vivax*. The relationship between the number of sporozoites injected and various measures of severity (unadjusted for any confounders).

		Number of sporozoites x 1000		
		< 30	30 - < 120	≥ 120
Peak parasitaemia (per 100 high power fields)	n	13	15	11
	Geometric mean	55.7	53.3	59.3
	95% CI	35.4-87.7	38.3-74.3	40.6-86.6
Pretreatment peak parasitaemia (per 100 high power fields)	n	13	15	11
	Geometric mean	50.1	47.1	56.9
	95% CI	26.2-96.0	31.4-70.6	40.2-80.6
Peak temperature (°F)	n	13	15	9
	Mean	104.9	105.2	105.3
	95% CI	104.7-105.1	104.8-105.6	104.9-105.8
Pretreatment peak temperature (°F)	n	6	8	4
	Mean	104.7	105.2	105.2
	95% CI	104.6-104.8	104.7-105.8	104.4-105.9
No. of peaks over 103°F	n	13	15	9
	Mean	9.23	9.53	10.33
	95% CI	7.69-10.77	7.75-11.32	9.06-11.61
No. of peaks over 103°F pretreatment	n	6	8	4
	Mean	7.00	8.63	9.25
	95% CI	4.61-9.39	6.58-10.67	3.99-14.51
No. of peaks over 103°F divided by time to treatment	n	13	15	9
	Mean	0.87	1.19	1.14
	95% CI	0.62-1.12	0.87-1.51	0.80-1.49
No. of peaks over 103°F pretreatment divided by time to treatment	n	6	8	4
	Mean	0.53	0.90	0.73
	95% CI	0.32-0.75	0.69-1.11	0.52-0.93
Days between patent parasitaemia and treatment	n	13	15	11
	Mean	12.31	9.27	10.09
	95% CI	8.49-16.13	8.49-11.89	7.81-12.37

Table 3.3.17. *P vivax*. The relationship between the prepatent period and various measures of severity in sporozoite-induced infections (unadjusted for any confounders).

		Prepatent period (days)		
		< 12	12 - 13	≥ 14
Peak parasitaemia (per 100 high power fields)	n	17	21	19
	Geometric mean	57.4	51.8	73.0
	95% CI	36.3-90.8	40.0-67.0	53.2-100.1
Pretreatment peak parasitaemia (per 100 high power fields)	n	17	21	19
	Geometric mean	46.9	50.1	61.5
	95% CI	27.1-81.0	38.8-64.7	47.1-80.3
Peak temperature (°F)	n	15	21	18
	Mean	105.1	105.2	105.1
	95% CI	104.8-105.6	104.9-105.5	104.9-105.4
Pretreatment peak temperature (°F)	n	6	12	13
	Mean	104.8	105.2	105.1
	95% CI	104.6-105.0	104.8-105.6	104.9-105.4
No. of peaks over 103°F	n	15	20	18
	Mean	9.87	9.20	10.39
	95% CI	8.50-11.24	7.85-10.55	9.02-11.76
No. of peaks over 103°F pretreatment	n	6	12	13
	Mean	8.50	8.33	8.77
	95% CI	4.48-12.52	6.92-9.75	7.21-10.33
No. of peaks over 103°F divided by time to treatment	n	15	20	18
	Mean	1.16	1.00	1.06
	95% CI	0.88-1.44	0.80-1.20	0.79-1.32
No. of peaks over 103°F pretreatment divided by time to treatment	n	6	12	13
	Mean	0.73	0.79	0.83
	95% CI	0.45-1.01	0.66-0.93	0.66-1.01
Days between patent parasitaemia and treatment	n	17	21	19
	Mean	9.41	10.10	12.00
	95% CI	8.04-10.79	8.03-12.16	9.24-14.76

Table 3.3.18. The relationship between the estimated number of sporozoites injected and various measures of severity (unadjusted for any confounders).

		Number of sporozoites x 1000		
		< 30	30 - < 120	≥ 120
Episodes of tertian fever	n	5/13	6/15	6/11
	%	38.5	40.0	54.5
Episodes of tertian fever pretreatment	n	2/11	1/14	1/8
	%	18.2	7.1	12.5
Spontaneous recovery	n	2/11	1/15	1/9
	%	15.4	6.7	11.1
Modifying treatment given	n	8/13	11/15	7/11
	%	61.5	73.3	63.6
At least 2 doses modifying treatment given	n	2/13	5/15	2/11
	%	15.4	33.3	18.2

Table 3.3.19. *P vivax*. The relationship between the prepatent period and various measures of severity in sporozoite-induced infection (unadjusted for any confounders).

		Prepatent period (days)			χ^2 linear trend	*p*
		< 12	12 - 13	≥ 14		
Episodes of tertian fever	n	7/17	10/21	6/19		
	%	41.2	47.6	31.6		
Episodes of tertian fever pretreatment	n	0/12	4/21	3/16		
	%	0.0	19.0	18.8	1.7	0.2
Spontaneous recovery	n	2/15	4/21	2/18		
	%	13.3	19.0	11.1		
Modifying treatment given	n	10/17	16/21	10/19		
	%	58.8	76.2	52.6		
At least 2 doses modifying treatment given	n	2/17	6/21	3/19		
	%	11.8	28.6	15.8		

Table 3.3.20. Summary of relationships of dose and prepatent period with major outcome measures from the analyses of induced malaria. All the results are from non-immune patients not treated within the first 5 days of patent parasitaemia.

0 = No relationship
+ = positive association (or outcome more common after higher doses or longer prepatent periods)
+ = $p < 0.05$, ++ = $p < 0.001$
- = negative association (or outcome less common after higher doses or longer prepatent periods)
- = $p < 0.05$, -- = $p < 0.001$

	P vivax Mosquitos one day (n = 289)		*P vivax* Mosquitos 2-3 days (n = 110)		*P vivax* Trophozoites (n = 107)		*P vivax* Sporozoites (n = 57)		*P ovale* Trophozoites (n = 80)		*P falciparum* Mosquitos (n = 82)	
	Dose[1]	Prepatent period	Dose[1]	Prepatent period	Dose	Prepatent period	Dose	Prepatent period	Dose	Prepatent period	Dose	Prepatent period
Pretreatment peak parasitaemia	0	0	+	0	0	0	0	0	0	0	0	0
Pretreatment peak temperature	0	0	0	0	0	0	0	0	0	- (0)[2]	0	0
Peaks over 103°F pretreatment	0	0	0	0	0	0	0	0	0	-	0	0
Modifying treatment	0 (+)	-- (-)	-	-	0 (-)	0	0	0			0	- / 0[3]
Spontaneous recovery	0	++	0	0	-	0	0	0	0	0		
Tertian fever pretreatment	0	++	0	0	0	+	0	+ (0)	0	0		

[1] Estimated number of infected mosquitos
[2] Result after adjusting for confounding (given only if it differs from crude result)
[3] Two different strains. The results refer to early treatment in this data set

Table 3.3.21. *P ovale.* The relationship between the prepatent period and the number of peaks over 103°F in trophozoite-induced ovale malaria

		No. of peaks over 103°F					
		Overall			Spontaneous recovery		
		n	mean	95% CI	n	mean	95% CI
Prepatent period (days)	≤ 10	25	7.28	6.09-8.47	20	7.45	6.01-8.89
	10-13	27	4.63	3.63-5.64	18	4.83	3.49-6.18
	> 13	27	4.85	3.45-6.16	18	4.94	3.41-6.47

Table 3.3.22. *P ovale.* The relationship between prepatent period and the duration of infection among patients with spontaneous recovery following trophozoite inoculation.

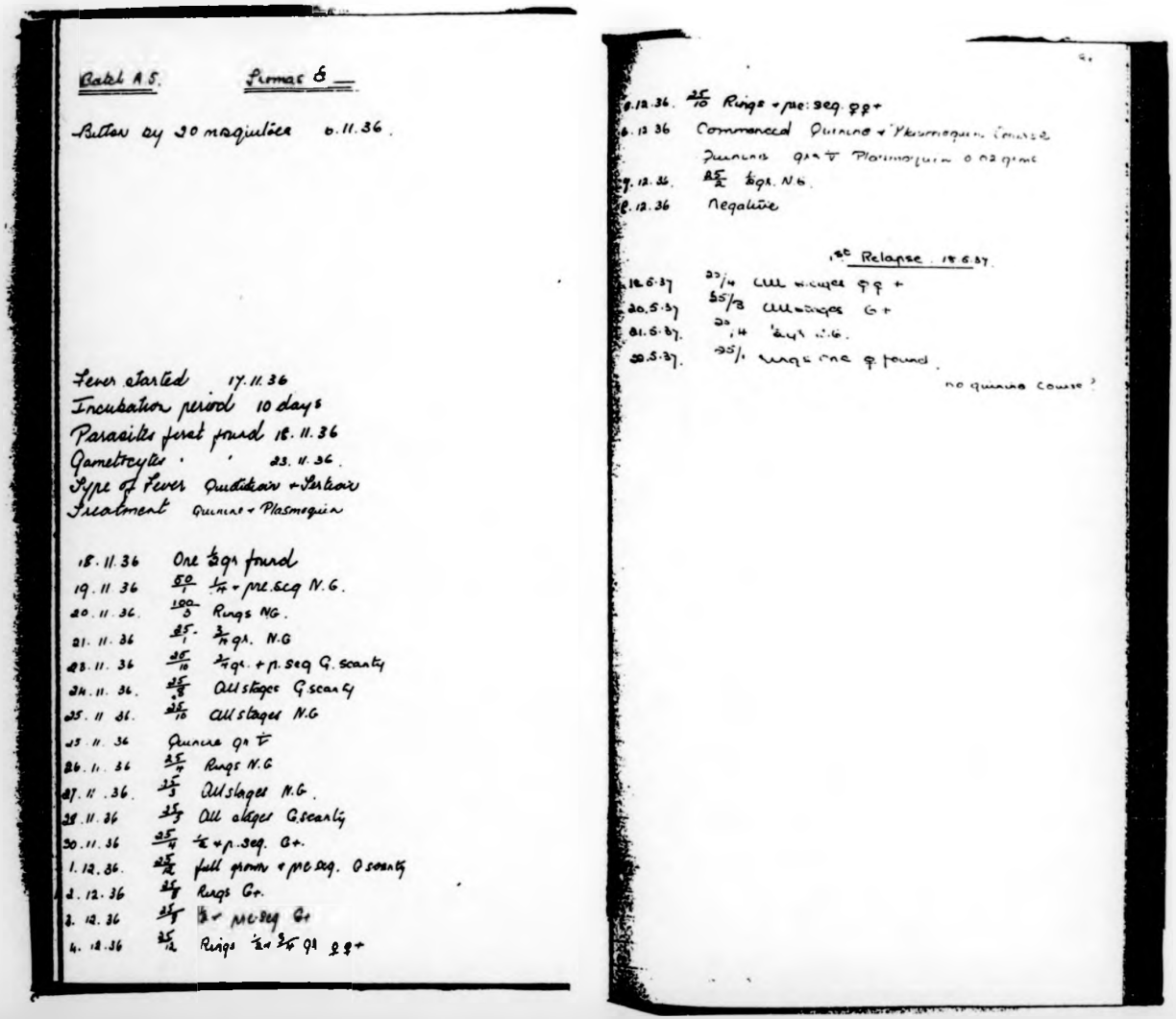
		Duration of infection (days)		
		n	Geometric mean	95% CI
Prepatent period (days)	≤ 10	20	20.0	16.8-23.7
	10-13	19	18.2	15.7-21.2
	> 13	18	13.7	11.4-16.4

Table 3.3.23. *P falciparum.* Variation between the strains of falciparum malaria used.

Strain	Number of mosquitos Geometric mean			Prepatent period Geometric mean			Geometric mean count over first 20 days		
	n	Mean	95%CI	n	Mean	95%CI	n	Mean	95%CI
EL	50	23.5	16.0-34.5	50	10.4	9.8-10.9	12	2250	965-5291
ML	38	10.4	8.0-13.5	39	12.9	12.0-14.0	16	549	260-1161
SC	10	20.6	8.7-48.5	10	9.7	8.2-11.5	9	1205	344-4224

Table 3.3.24. *P falciparum.* Types of mosquitos used in induced falciparum malaria

Mosquito species	Strain					
	EL		ML		SC	
	Early treatment		Early treatment		Early treatment	
	No	Yes	No	Yes	No	Yes
An albimanus	12	29	2	1	1	-
An quadrimaculatus	-	4	13	21	8	1
An crucians	-	-	1	-	-	-
An freeborni	-	3	-	-	-	-
mixed	-	2	-	-	-	-



Batch A.S. Primar 6

Bitten by 30 mosquitoes 6.11.36.

Fever started 17.11.36
Incubation period 10 days
Parasites first found 18.11.36
Gametocytes " " 23.11.36
Type of Fever Quotidian + Tertian
Treatment Quinine + Plasmoquin

18.11.36 One ♀gr found
19.11.36 $\frac{80}{1}$ $\frac{1}{11}$ + pre.seq N.G.
20.11.36. $\frac{100}{3}$ Rings NG.
21.11.36 $\frac{25}{1}$ $\frac{3}{11}$ gr. N.G
22.11.36 $\frac{25}{10}$ $\frac{2}{11}$ gr. + p.seq G. scanty
24.11.36. $\frac{25}{8}$ All stages G scanty
25.11.36. $\frac{25}{10}$ All stages N.G
25.11.36 Quinine gr T
26.11.36 $\frac{25}{7}$ Rings N.G
27.11.36. $\frac{25}{3}$ All stages N.G.
28.11.36 $\frac{25}{9}$ All stages G.scanty
30.11.36 $\frac{25}{4}$ ♂ + p.seq. G+.
1.12.36. $\frac{25}{10}$ full grown + pre seq. G scanty
2.12.36 $\frac{25}{9}$ Rings G+.
3.12.36 $\frac{25}{9}$ ♂ + pre.seq G+
4.12.36 $\frac{25}{12}$ Rings ♂ & $\frac{2}{25}$ gr ♀ ♀+

4.

4.12.36. $\frac{25}{10}$ Rings + pre: seq. ♀♀+
6.12.36 Commenced Quinine + Plasmoquin (mixture)
Quinine gr x T Plasmoquin 0.02 grms
7.12.36. $\frac{25}{2}$ ♀gr. N.G.
8.12.36 Negative

1st Relapse 18.6.37.

18.6.37 $\frac{23}{4}$ All stages ♀♀ +
20.6.37 $\frac{25}{3}$ All stages G +
21.6.37. $\frac{20}{4}$ ♀gr N.G.
22.6.37. $\frac{25}{1}$ Rings one ♀ found.
no quinine course?

Figure 3.3.1. Example of malaria therapy entry from first set of books

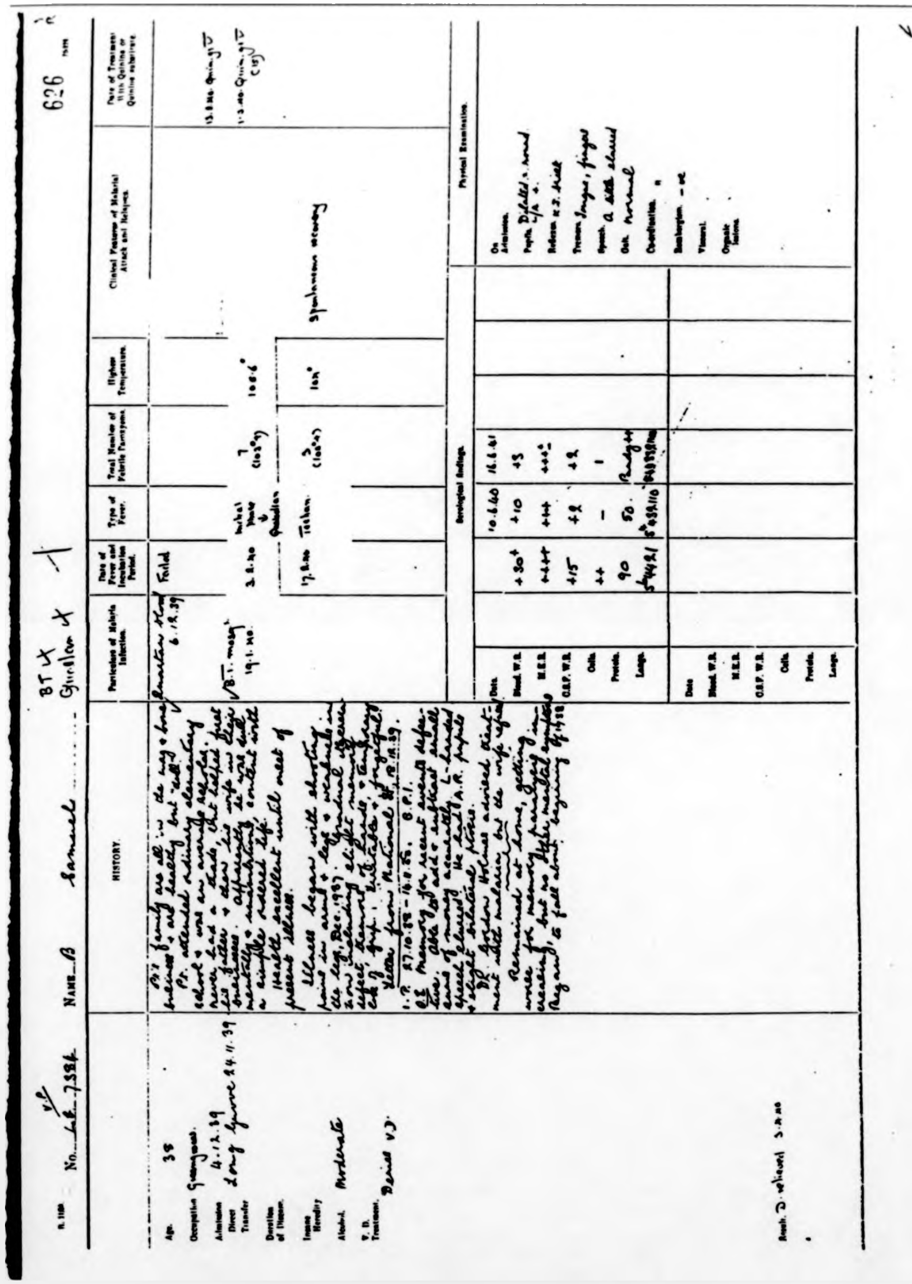


Figure 3.3.2. Example of an entry from the second set of books.

6.

TREATMENT RECORD.

17.10.58 Bitten by B.T. mosquitoes.

Particulars of Malaria Therapy B.T.

17.10.58. Bitten by B.T. mosquitoes. Fever began 31.10.58 and was at first irregular. Then quotidian. 7.11.58. had Thio. Bismuth 0.2 gms. intramuscularly. This was followed by 24 hrs remission and tertian fever. 16.11.58. had Thio. Bismuth 0.2 gms. intramuscularly. which was followed by 72 hrs remission. 20.11.58 began a Course of Quin. gr x daily for 7 days. Had 8 peaks of 103°F & over. H.T. 105.4°F.

Particulars of 1st B.T. Relapse

Fever began 9.12.58 and was quotidian. 12.12.58 had Thio. Bismuth 0.2 gms. intramuscularly and became afebrile 13.12.58 Fever recurred 22.12.58 and was tertian. 23.12.58. had Thio. Bismuth 0.2 gms. intramuscularly. becoming afebrile 24.12.58 Fever recurred 2.1.59 and ended in a Spontaneous recovery after two tertian peaks. 13.1.59. began a Course of Quin. gr x daily for 7 days. Had 2 3 peaks of 103°F and over. H.T. 105.2°F.

Figure 3.3.3. Example of description of malaria therapy in a patient's medical notes.

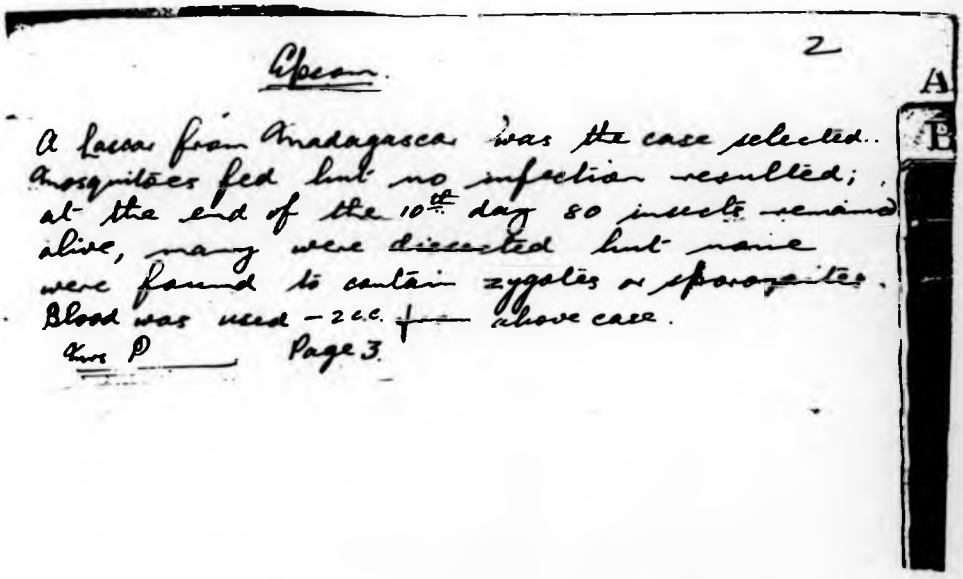
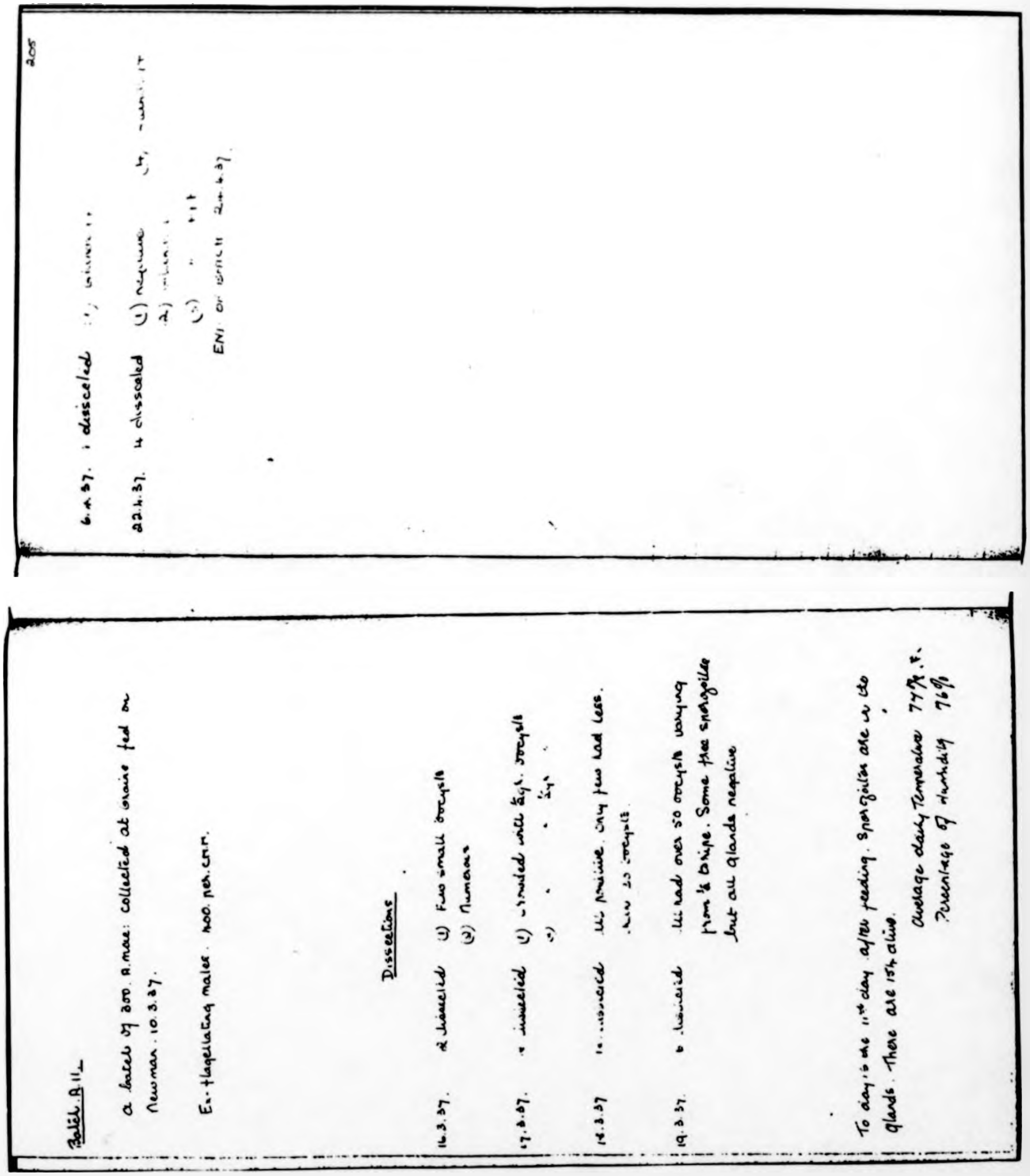


Figure 3.3.4. Decription of the origin of the Madagascar strain. From first set of books.


Batch. A.11.

a batch of 300. A.mac: collected at ... fed on Newman. 10.3.37.

Ex-flagellating males. 400. per. c.m.

Dissections

16.3.37. 2 dissected (1) Few small oocysts
(2) Numerous

17.3.37. ... dissected (1) crowded with ... oocysts

18.3.37 10 ... dissected All positive. Only few had less ... 20 oocysts.

19.3.37. 6 dissected All had over 50 oocysts varying from ... Some free sporozoites but all glands negative

To day is the 11th day after feeding. Sporozoites are in the glands. There are 154 alive.
Average daily Temperature 77°F.
Percentage of humidity 76%

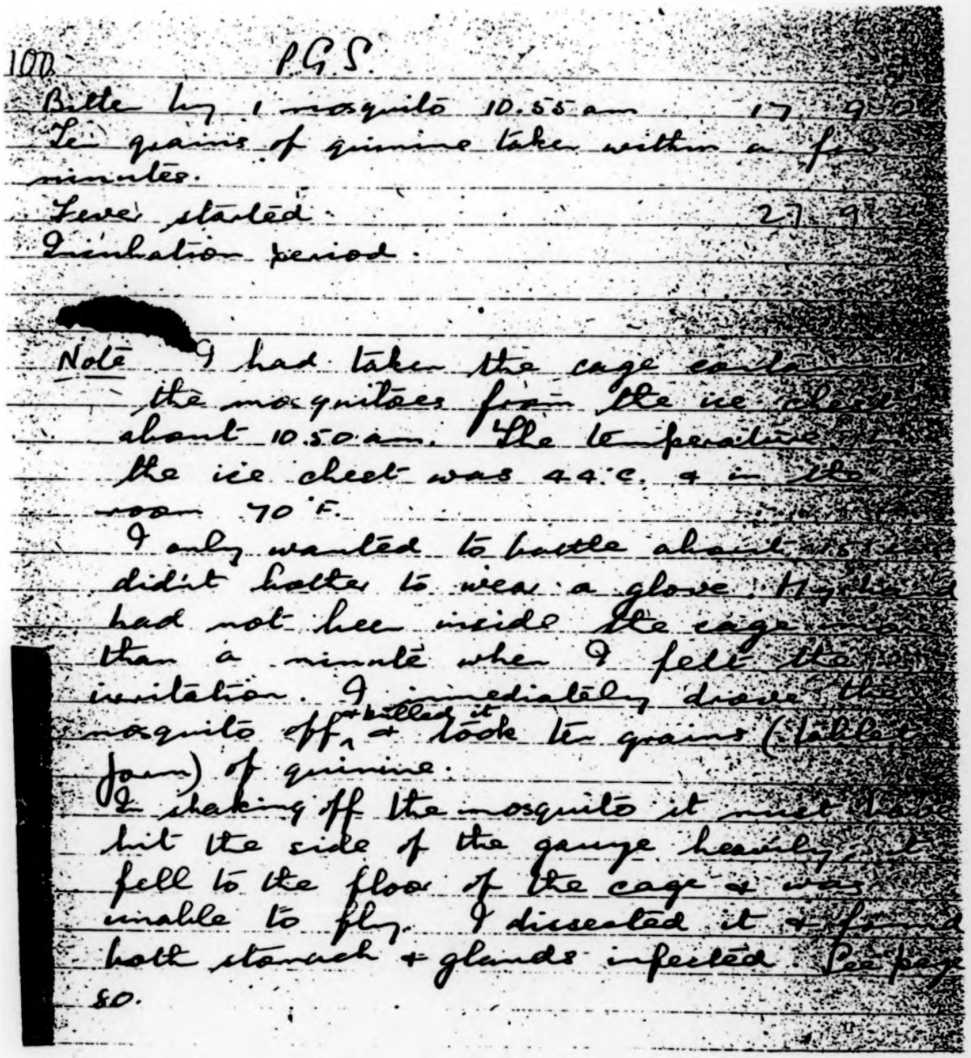
205

6.4.37. 1 dissected ...

22.4.37. 4 dissected (1) negative ...

END OF BATCH 24.4.37.

Figure 3.3.5. Description of a batch of mosquitos used for malaria therapy. From first set of books.


100 P.G.S.
Bitten by 1 mosquito 10.55 am 17 9
½ grains of quinine taken within a few minutes.
Fever started 27 9
Incubation period:

[redacted]

Note I had taken the cage containing the mosquitoes from the ice chest about 10.50 a.m. The temperature in the ice chest was 44°C. & in the room 70°F.
I only wanted to bottle about 10 & didn't bother to wear a glove. My hand had not been inside the cage more than a minute when I felt the mosquito biting. I immediately drove the mosquito off, killing it & took ½ grains (tablets) of quinine.
In shaking off the mosquito it must have hit the side of the gauze heavily as it fell to the floor of the cage & was unable to fly. I dissected it & found both stomach & glands infected. See page 80.

Figure 3.3.6. An entry mentioning the use of an ice chest for storing mosquitos. (It describes the accidental infection of PG Shute.)

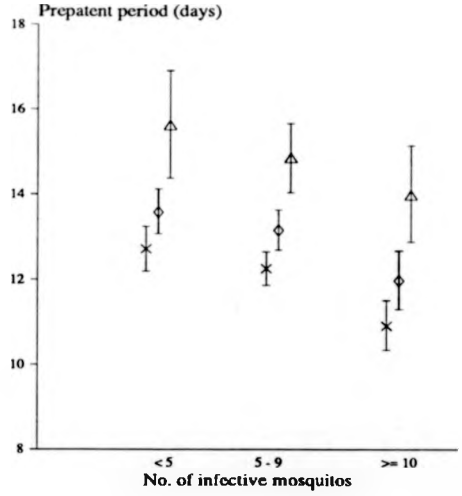


Figure 3.3.7. *P vivax.* Geometric mean prepatent periods (with 95% CI) by the estimated number of infected mosquitos and the storage time, among non-immune patients bitten on only one day. × = stored < 10 days, ◊ = stored 10-19 days, ▵ = stored ≥ 20 days.

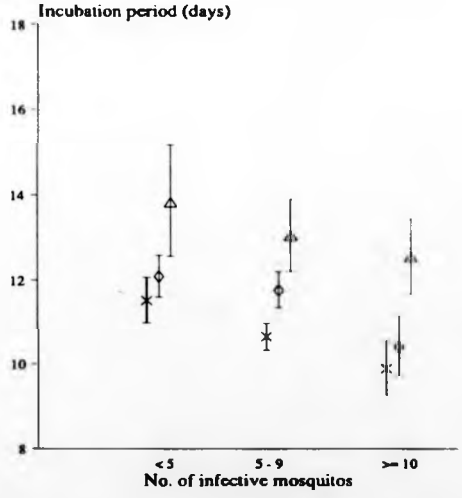
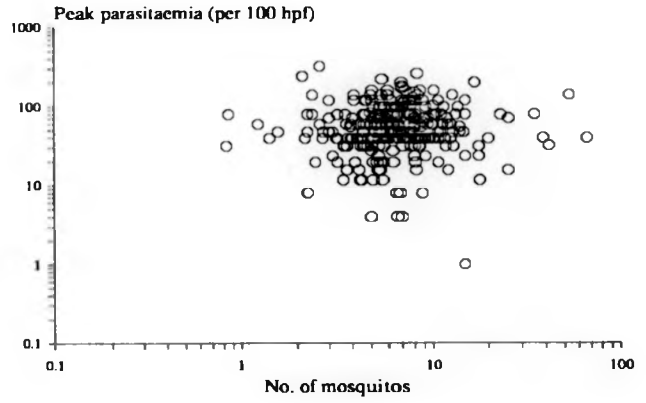


Figure 3.3.8. *P vivax.* Geometric mean incubation periods (with 95% CI) by the estimated number of infected mosquitos and the storage time, among non-immune patients bitten on only one day. × = stored < 10 days, ◊ = stored 10-19 days, ▵ = stored ≥ 20 days.



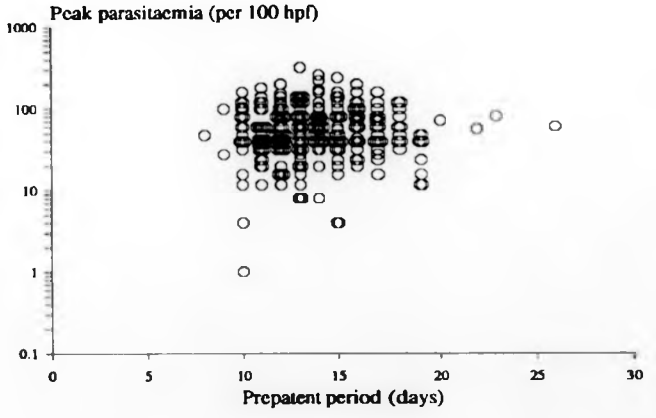


Figure 3.3.9. ***P vivax*****. The relationships between the estimated number of infected mosquitos, and prepatent period with the pretreatment peak parasitaemia, among non-immune patients bitten on only one day.**

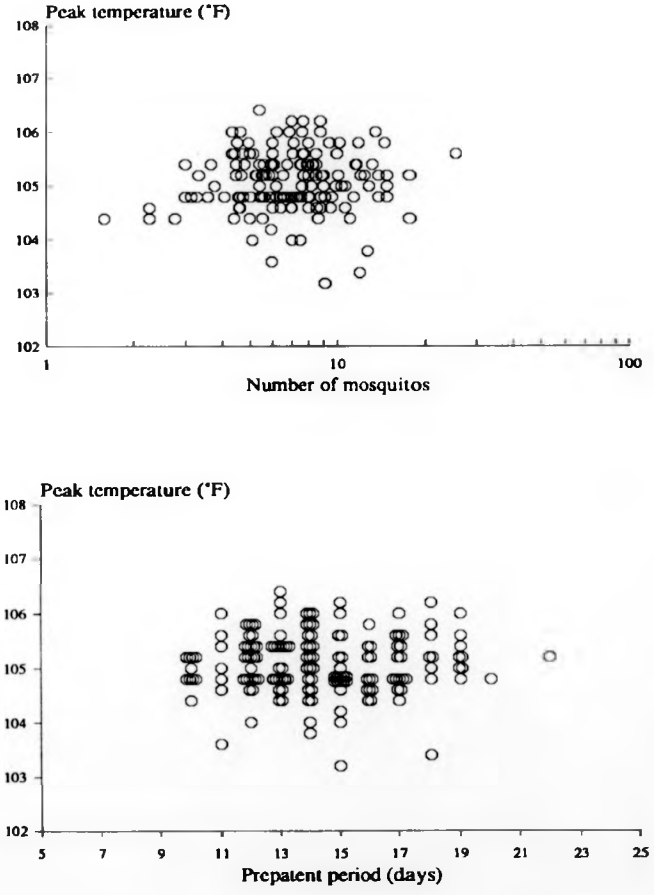


Figure 3.3.10. ***P vivax.*** **The relationships between the estimated number of infected mosquitos, and prepatent period with the pretreatment peak temperature, among non-immune patients bitten on only one day.**

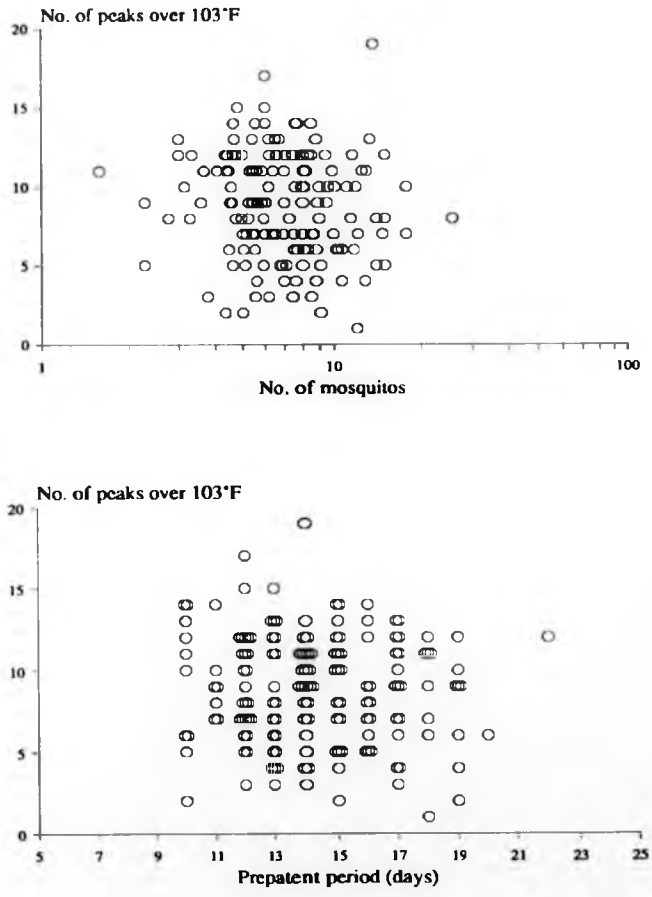


Figure 3.3.11. ***P vivax.*** **The relationships between the estimated number of infected mosquitos, and prepatent period with the number of peaks over 103°F pretreatment, among non-immune patients bitten on only one day.**

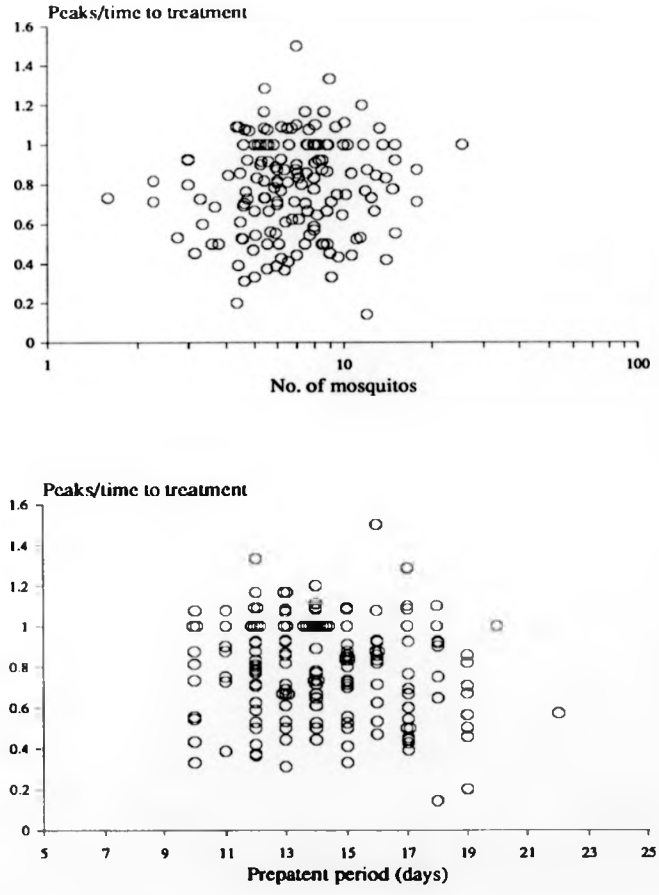
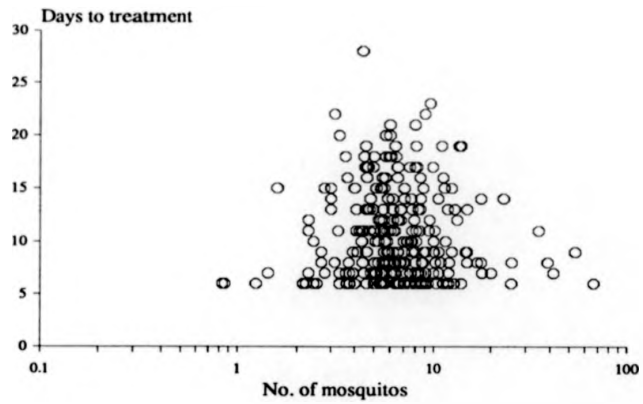


Figure 3.3.12. ***P vivax.*** **The relationships between the estimated number of infected mosquitos, and prepatent period with the number of peaks over 103°F pretreatment as a proportion of the number of days between patent parasitaemia and treatment, among non-immune patients bitten on only one day.**



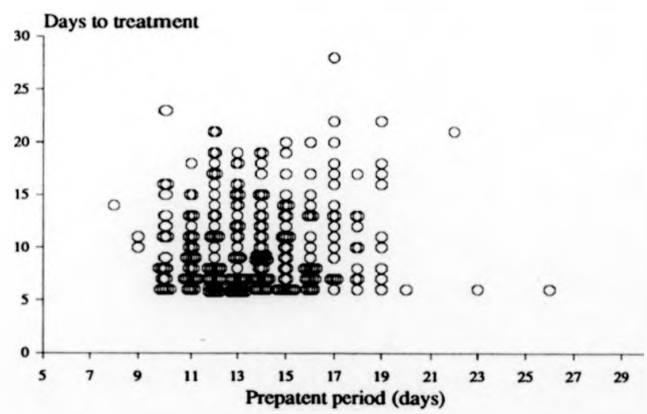


Figure 3.3.13. ***P vivax.*** **The relationships between the estimated number of infected mosquitos, and prepatent period with the number of days between patent parasitaemia and treatment, among non-immune patients bitten on only one day.**

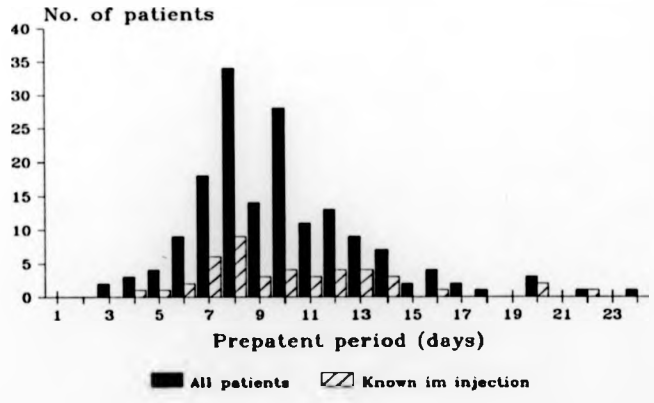


Figure 3.3.14. *P vivax*. Prepatent periods in trophozoite-induced malaria (selected as in text).

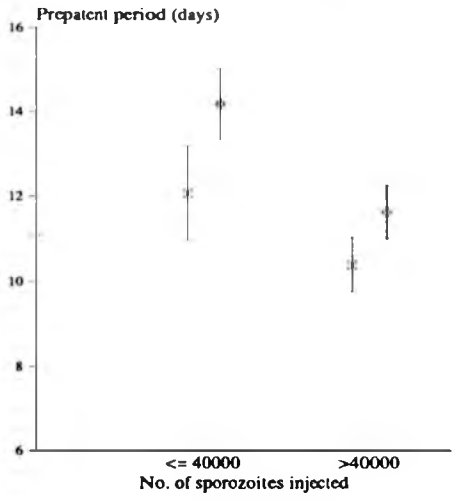


Figure 3.3.15. *P vivax*. Mean prepatent periods (with 95% CI) by the sporozoite number and storage time, among non-immune patients. × = stored for < 20 days, ◊ = stored ≥ 20 days.

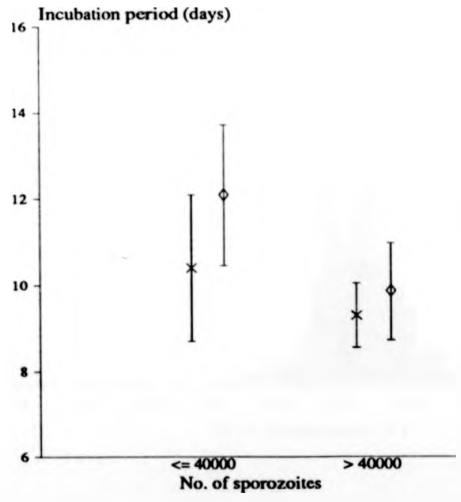


Figure 3.3.16. *P vivax*. Mean incubation periods (with 95% CI) by sporozoite number and storage time, among non-immune patients. × = stored < 20 days, ◊ = stored ≥ 20 days.

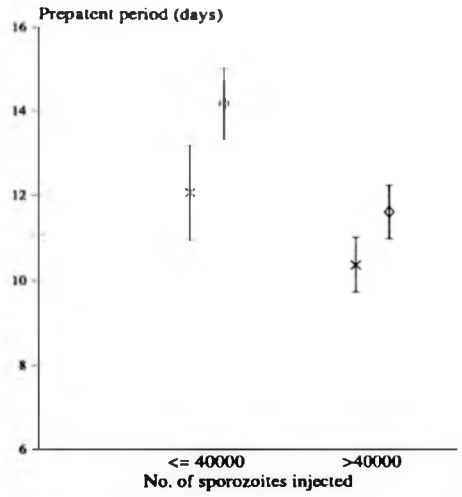


Figure 3.3.15. *P vivax*. Mean prepatent periods (with 95% CI) by the sporozoite number and storage time, among non-immune patients. × = stored for < 20 days, ◊ = stored ≥ 20 days.

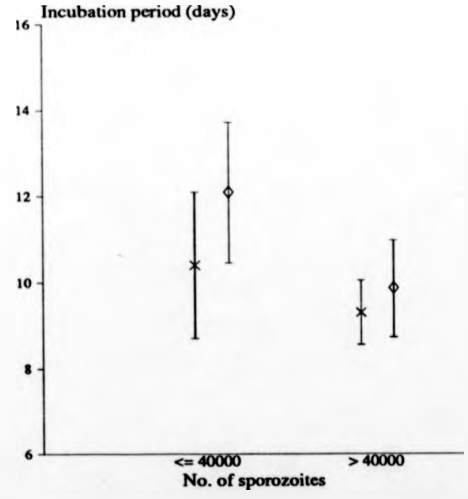


Figure 3.3.16. *P vivax*. Mean incubation periods (with 95% CI) by sporozoite number and storage time, among non-immune patients. × = stored < 20 days, ◊ = stored ≥ 20 days.

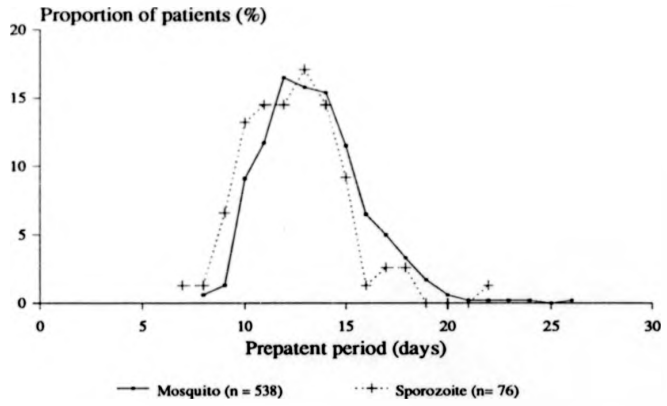


Figure 3.3.17. *P vivax*. Comparison of prepatent periods for mosquito-induced and sporozoite-induced malaria.

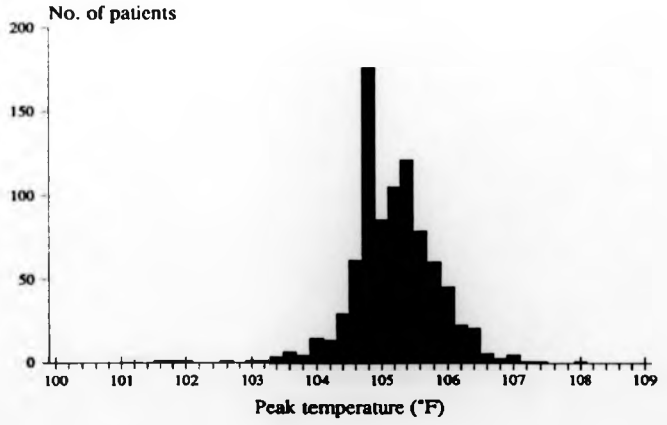


Figure 3.3.18. *P vivax*. Peak temperature reached in mosquito-induced malaria in non-immune patients.

CONCLUSIONS

Relationship of the findings to the pathogenesis of malaria

Overall, very little evidence of an association between dose and severity was found for malaria (Table 3.3.20). Most of the correlations that were found used prepatent or incubation period as markers of dose, and so these associations could be due to confounding by susceptibility.

From theoretical knowledge of the pathogenesis of malaria a dose-severity relationship might be predicted. Observations on patients show that in the early stages of infection parasite numbers closely follow the predicted logarithmic increase (MaeGraith 1948). A smaller inoculum size should multiply at the same rate and follow a parallel curve to a larger one, reaching a threshold level at a correspondingly later time. Hamilton Fairley's sub-inoculation experiments demonstrated that this occurs in practice, (Hamilton Fairley 1947) and the time taken to reach the level where the parasitaemia is just detectable - the prepatent period - has been seen to depend on dose. After a given time, during this stage of initial multiplication, the parasite load will be smaller after small inocula than after large ones. Since the immune response is time-dependent, after smaller inocula it will start to take effect while the parasite load is lower, and should therefore be more effective in limiting the severity of disease (Greenwood et al 1991, Marsh 1992). In this context, the failure to detect an association between dose and severity is perhaps surprising. How can this lack of association be explained?

It is useful to consider what is happening in two stages. The first relates the dose to the level of parasitaemia attained, and the second relates the parasitaemia to the other measures of severity. If parasitaemia is a poor predictor of severity then any relationships with the initial dose will be intuitively less likely.

The failure to find associations at the first stage, between dose and the maximum parasitaemia reached, is perhaps the most unexpected. However, the relationships between the measured infecting dose and the true dose and the measured blood parasitaemia and the true parasite load are both imprecise, in my analyses and in the published studies. The reasons for this have already been addressed. This imprecision causes non-differential misclassification and therefore underestimation of any association. In only one of the data sets examined (mosquito-induced vivax malaria where mosquitos were used over 2-3 days) was any relationship between dose

and peak parasitaemia found. In none was a relationship between prepatent or incubation period and peak parasitaemia demonstrated, even though confounding by susceptibility would be expected to lead to such an association. A strong correlation between dose and peak parasitaemia seems unlikely.

In an individual person, changing levels of parasitaemia are reflected in the fever and associated symptoms (Kitchen 1949). Between individuals, associations between parasitaemia and other measures of severity are also found but are less clear (Lowe 1934). In patients with chronic infections parasitaemias are higher in periods of fever than in periods without (Ross & Thomson 1910, Sinton et al 1931). Among schoolchildren in the Congo where around 80% have detectable parasitaemias, those thought clinically to have malaria had higher parasite densities than those thought to have other diseases or asymptomatic controls (Trape et al 1985). Among malaria patients, correlations have been found between the parasitaemia and the peak temperature (Gillespie et al 1991) and the proportion of patients with high temperatures (Ejezie & Ezedinachi 1992). In induced malaria, the maximum parasite density has been related to the duration of the clinical attack (Boyd 1938). In natural falciparum malaria, very high parasitaemias have been associated with increased case fatality rates (Field & Niven 1937) and poor outcome from cerebral malaria (Molyneux et al 1989). However the relationships are not consistent (Walker et al 1992, Kwiatowski et al 1990, Grau et al 1989), and even where an association is demonstrated at very high parasitaemia levels, the similarity in the overall distributions of presenting parasitaemias among those with full recovery and those with poor outcome is striking (Molyneux et al 1989). Some of the variation in falciparum malaria can be explained by the tremendous fluctuations in blood parasitaemia levels which occur within 24 hours, but this is much less of a problem with vivax (Lowe 1934).

Tumour necrosis factor (TNF) and interleukin-1α levels are higher in severe malaria, and correlate with parasitaemia levels (Kwiatowski et al 1990, Grau et al 1989, Shaffer et al 1991). Within an individual the fever closely follows changes in TNF levels (Karunaweera et al 1992). Higher parasitaemias have also been associated with increases in acute phase proteins (Gillespie et al 1991) and changes in haematological factors towards a procoagulant state (Hemmer et al 1991).

In induced malaria it is possible to measure the number of parasites associated with the first temperature (the pyrogenic threshold). Taking 100°F (37.8°C) as the cut-off, Boyd (1938, 1940c, 1944b) found considerable variation in the initial pyrogenic threshold, with a range

from undetectable to over 1000 parasites per μl (median 10-50) for vivax (Boyd 1938). Longer incubation periods were associated with higher parasite densities. The parasite count associated with the first temperature of 104°F was also highly variable (Boyd 1944b) but in nearly 90% of patients the parasitaemia on the day of the first temperature of 104°F was higher than that on the day of the first temperature of 100°F. During the infection the tolerance of the patient increases, so that the last fever of over 100°F in the attack was associated with much higher parasitaemias (median level of 2000-4000 per μl) than those found at the time of the first fever of 100°F (Boyd 1938).

The picture is of a trend towards more severe disease at higher parasitaemia levels but with considerable variation. Both the results from natural infections and Boyd's reports of induced malaria are likely to be confounded by variations between different strains of malaria and in immunity of the patients. (Boyd's observations pool the results of more than one strain of malaria and may include some semi-immune patients, although this is not mentioned.)

In the Horton data the analysis has been restricted to single strains and non-immune patients. The relationships between the pretreatment peak parasitaemia and various measures of severity are summarised in Table 3.4.1. For the 80 patients infected with *P ovale* the peak parasitaemia was correlated with the peak temperature, with the number of peaks over 103°F, and with the duration of the infection. There was no correlation with the type of fever or whether spontaneous recovery was recorded.

For Madagascar strain vivax malaria correlations were found with the number of peaks over 103°F but not with the peak temperature. Modifying treatment was associated with lower peak parasitaemias; it may be that the treatment prevented the development of higher parasitaemias.

For the American falciparum data no correlations were found between the pretreatment peak parasitaemia and the peak fever but for one of the two main strains the number of peaks over 103°F increased with the peak parasitaemia.

Overall there is some evidence of a relationship between parasitaemia and other measures of severity, but the associations are generally weak, and, although some of the lack of association can be attributed to inaccuracies of measurement, as described above, these results emphasise the variability in response even of non-immune individuals given a single strain of malaria. There is very little evidence for a relationship between dose and parasitaemia. The lack of

consistent associations between direct measures of dose and measures of severity, while marred by inaccuracies of measurement, suggests that any true effect of dose is unlikely to be strong in comparison to all the other causes of variation in severity of disease between individuals.

Implications for natural malaria

The finding that insecticide-impregnated bednets decrease symptomatic malaria and death more than infection has been interpreted as providing evidence that dose influences severity of disease in malaria (Snow et al 1988, Greenwood et al 1991, Bermejo and Veeken 1992). The more direct evidence discussed above suggests that a strong dose-severity effect is unlikely, but little of it relates to falciparum malaria.

What does "infecting dose" mean?

In terms of natural malaria what is meant by infecting dose? The dose which we want to consider is the number of sporozoites inoculated that can be associated with a particular episode of malaria. This will vary with the number of sporozoites transmitted per mosquito and with the frequency of mosquito bites. The period of time over which the doses from separate infective bites can be considered to add together to constitute a single infective dose is not known. Mosquitos appear to transmit a median of less than 20 sporozoites, (Rosenberg et al 1990, Beier et al 1991a,b, Ponnundurai et al 1991). Multiplication in the liver increases parasite numbers in the order of 2000-40000 times, depending on species (McGregor 1965). The liver cycle is a fixed length, so parasites embarking on it two days later than parasites from a previous bite will, on average, be released as merozoites two days later. By this time the progeny of the first bite will have been through a cycle of erythrocytic multiplication (for falciparum, vivax and ovale malaria) and so will have multiplied a further 16-24 times (Garnham 1988). These parasites are therefore likely to outnumber the newcomers unless the number of sporozoites from the second bite was much greater than the first. The time period over which the sporozoites from separate bites can be considered to add to form a single infective dose is therefore likely, on average, to be less than the length of one or two erythrocytic cycles. Therefore, in most situations, episodes are likely to arise from a single bite.

In endemic areas where most of the population have some level of parasitaemia most of the time, the incoming number of parasites associated with being rebitten is small compared with the total body load (Lines & Armstrong 1992). Nevertheless, there is evidence that new infections do trigger disease in these conditions. In The Gambia, parasitaemia prevalence rates

decrease only slightly during the dry season, but the clinical malaria and mortality from malaria occur almost entirely during the wet season (Greenwood et al 1987, Alonso et al 1991). Controlling mosquito transmission by using impregnated mosquito nets - that is, preventing some new infective bites - decreases clinical episodes and mortality (Snow et al 1987, 1988, Alonso et al 1991). If new inocuations trigger disease more readily than the parasites already in the host, the host must be less able to limit the multiplication of the new parasites than of the old ones. This would occur if immunity is strain specific and the new parasites were of a different strain. It would still be possible, in theory, for the inoculum size (of the new strain) to influence severity (Lines & Armstrong 1992).

How could impregnated bednets influence the infecting dose?

Impregnated bednets could decrease the infecting dose per episode by decreasing the frequency of infected bites and therefore the number of infective bites per episode and/or by decreasing the number of sporozoites transmitted per mosquito. It has been suggested that impregnated nets may prevent a mosquito from feeding normally and from transmitting a full dose of sporozoites (Snow et al 1988, Greenwood et al 1991). Whether this occurs is important but unknown. If the frequency of infectious bites is low enough that most cases of malaria are likely to arise from the bite of one infected mosquito, then the effects of the intervention would be to decrease the chance of that single bite. Only if the intervention also influences the number of sporozoites inoculated per mosquito would the dose per episode be affected. Even in areas with a high prevalence of malaria, only a proportion of infected mosquitos would carry "new" strains, and most cases of malaria might still arise from the bite of a single mosquito. In this case, unless the intervention decreased the dose delivered per mosquito then it would have no effect on the dose per episode.

Difficulties in interpreting the results of bednet studies

If bednets reduce the "infecting dose" and dose determines whether infection or disease results, then dose-effects could explain the disproportionate decrease in disease and asymptomatic infection seen in bednet studies. Dose is not, though, the only possible explanation. The disproportion may arise quite simply from saturation. As the frequency of infection-causing bites increases, a greater proportion fall on already-infected people; the same happens with disease-causing bites, but since these are rarer, the degree of saturation is less at any given transmission intensity. The resulting disproportion in protective efficacies against the different outcomes can be illustrated with a numerical example.

Suppose, during the study period, those without nets are bitten by a mean of 2 infected mosquitos and those with nets with a mean of 1 infected mosquito and that the probability of the outcome per bite is 90% for parasitaemia, 50% for symptoms and 1% for severe symptoms. Assume that the chance of being bitten and of a particular outcome occurring are random processes. The mean number of events per person (m) is then:

	Mean events per person per study period	
	Without net	With net
Parasitaemia	1.8	0.9
Symptoms	1.0	0.5
Severe symptoms	0.02	0.01

The chance of not getting a random (Poisson) event of mean m is e^{-m}, so the chance of an event occurring is $1 - e^{-m}$. The probability per person of each outcome during the study period is then:

	Probability of outcome per person per study period (%)	
	Without net	With net
Parasitaemia	83.47	59.34
Symptoms	63.21	39.35
Severe symptoms	1.98	1.00

In a population these probabilities are equivalent to the proportion of people with each outcome, and the protective efficacy of bednets against each outcome can be calculated as (1 - relative risk). The protective efficacy is 28.9% against parasitaemia, 37.7% against symptoms and 49.5% against severe symptoms.

The calculations can be modified to take account of different probabilities of the various outcomes in different groups of the population, or to incorporate more than one strain of malaria. The general principle that the protective efficacy is greater against the less common outcome remains.

These calculations are concerned only with the presence or absence of an event during the study period, assume that single infections and multiple infections are equally likely to give symptoms and ignore any changes in immunity during the study period. They also assume that bites are random although aggregation is likely. Even so they demonstrate that in the absence of a dose-severity relationship, the protective efficacy cannot be assumed to be the same against all the different outcomes.

In a study of impregnated bednets in The Gambia, (Snow et al 1988) the protective efficacy against parasitaemia was 32% (95% confidence interval -14% to 60%). This was calculated from cross-sectional surveys either side of the transmission season. Impregnated bednets may decrease the rate of re-establishment of parasitaemia after it is artificially cleared at the start of a study, but if parasitaemia rates are only measured from comparison of cross-sectional surveys carried out too late, after all or most of the susceptibles are infected, this may be missed. In the same study the protective efficacy against febrile episodes associated with parasitaemia was found to be 70% (95% confidence interval 41% to 87%) but this was calculated from incidence rates derived from weekly morbidity surveillance, so the two protective efficacies should not be directly compared.

So far we have assumed that the outcomes can be measured accurately, but defining malaria in the field is notoriously difficult. Using fever with parasitaemia as a defintion of mild malaria is likely to include fevers due to other causes. If this kind of misclassification occurs and is non-differential between intervention and control groups, this will lead to underestimation of the protective efficacy of the intervention. Misclassification is likely to be more of a problem for mild episodes than severe episodes. If the true protective efficacy were the same against mild and severe malaria, this difference in misclassification could give rise to an apparent but spurious greater efficacy against severe than total malaria. Misclassification may explain the results found in Kenya where, using weekly surveys for both symptoms and parasitaemia, the protection provided by impregnated bednets or curtains against new parasitaemias was greater than that against symptoms (Sexton et al 1990).

Confounding by age could also give rise to a greater decrease in severe than in mild malaria. Nets would be expected to be more effective in preventing bites among those who spend more time under them; this is likely to be the younger children, and younger children are more likely to get severe malaria. Socioeconomic status is also a potential confounder since it could influence both net use and outcome.

Conclusions

If there is a dose-severity relationship for malaria then in controlled trials of impregnated bednets the estimated protective efficacy against severe malaria might be expected to be greater than that against mild malaria, which in turn may be greater than that against parasitaemia. These trends have been found in some of the few studies which provide sufficient information on different outcomes, but explanations other than a dose-severity effect are possible.

On the basis of simple probability theory disproportionate decreases in symptomatic and asymptomatic malaria would be expected even without a dose-effect. These results may also arise due to other factors such as misclassification, confounding and study design. Dose may influence severity in malaria but the evidence from induced malaria suggests a strong relationship is unlikely. The results of bednet trials will not allow us to resolve the issue.

Table 3.4.1. The relationship between pretreatment parasitaemia and various measures of severity in the different data sets of induced malaria. All the results are from non-immune patients not treated within the first 5 days of patent parasitaemia

0 = No relationship
+ = positive association (or outcome more common with higher parasitaemia)
+ = $p < 0.05$, ++ = $p < 0.001$
- = negative association (or outcome less common with higher parasitaemia)
- = $p < 0.05$, -- = $p < 0.001$

	P vivax Mosquitos 1 day (n = 289)	*P vivax* Mosquitos 2-3 days (n = 110)	*P vivax* Trophozoites (n = 107)	*P vivax* Sporozoites (n = 57)	*P ovale* Trophozoites (n = 80)	*P falciparum* Mosquitos (n = 82)
Pretreatment peak temperature	0	0	0	0	++	0
Peaks over 103°F pretreatment	+	+ (0)[1]	+ (0)	+ (0)	++	+ / 0[2]
Modifying treatment	-	-	0	-		0
Spontaneous recovery	0	-	-	0	0	
Tertian fever pretreatment	0	0	0	0	0	

1. Result after adjusting for time to treatment (given only if it differs from the crude result)
2. Two different strains

SECTION 4

DISCUSSION

THE RELATIONSHIP BETWEEN INFECTING DOSE AND THE PATHOGENESIS OF DISEASE

> "Every infection is a race between the ability of the invading microbe to multiply and cause disease and the ability of the host to mobilise specific and non specific defences - a delay of a day or so on the part of the host can be critical." (Mims 1987)

A larger infecting dose of a microbe is more likely to reach "the critical levels of growth that give tissue damage and disease" (Mims 1987) before it can be limited by the immune response. This is reflected in the well known relationship for many infections between infecting dose and attack rate - higher doses giving higher attack rates. The idea of multiplication of the inoculum until a critical load is reached also explains the inverse relationship between dose and incubation period: larger inocula have a head start and so give rise to symptoms earlier. This simple relationship has been illustrated by Meynell (1963), see my Figure 4.1a).

It seems intuitively reasonable that the race should continue beyond the onset of illness and that therefore the head start given by higher doses should continue to have an effect. If one critical load determines the initial response, another higher one may determine a more severe response, and this second level may be reached more frequently by higher doses.

Models of microbial infection

The relationship between dose and outcome is likely to be more complex than this, and discussion of it has given rise to various models of microbial infection. The fact that more than one inoculated bacterium is usually required to produce a response or to kill a host can be explained in two different ways (Meynell & Stocker 1957). Either the bacteria could act co-operatively so that the response is the result of their joint action, or they could act independently but more than one is usually needed because the probability for any single bacterium of producing a response is less than one.

The extreme form of the first hypothesis gives rise to the concept of an individual effective dose - a dose above which a given host will certainly respond - and is therefore deterministic in nature (Armitage et al 1965). The second hypothesis, the "hypothesis of independent action" is stochastic and states that p, the mean probability per inoculated bacterium of multiplying sufficiently to produce a response, is independent of d, the number of bacteria inoculated (Meynell & Stocker 1957). Intermediate models involving partial synergism can also be defined, in which inoculated bacteria act independently at any given dose but where p increases

as d increases. Though possibly more realistic (Meynell & Meynell 1958) they have been studied less, perhaps because they can only be distinguished from complete independence experimentally if the hosts do not differ in resistance (Meynell & Stocker 1957).

Armitage et al (1965) subdivide stochastic models according to the assumed reason that p is less than one:

> 1) The culture from which the inocula are drawn contains a mixture of completely virulent and completely avirulent organisms so that p is actually the proportion of virulent organisms. This is thought to be unlikely.
>
> 2) The fate of an organism depends on the site it reaches after inoculation and p is the probability per inoculated organism of reaching the interior.
>
> 3) Both (1) and (2) imply that there is one decisive stage and that the heterogeneity of the host response is decided soon after inoculation. In contrast, the birth-death model assumes that "the outcome is determined by successive random events which continually operate as long as even one organism remains viable in its host". The model depends on the probabilities of organisms dividing and dying.

The models have been tested in various experimental systems, mostly bacterial infections of small rodents. The hypothesis of independent action predicts that the slope of the probit-mortality/log dose curve will be ≤ 2 at the LD50 point (Meynell & Stocker 1957). (The derivation is given by Peto (1953). It would equal 2 if the hosts have homogeneous resistance). This had been demonstrated using salmonellae in mice (Meynell & Stocker 1957, Meynell 1957), and is in contrast to the values greater than 2 estimated in experiments on dose-response of toxic chemicals and killed organisms, in which "co-operation" must be occurring (Armitage et al 1965).

Independent action also predicts that at low challenge doses, the resulting response may be the result of the multiplication of few, or sometimes one organism. For doses $\leq$ LD50 most fatally infected hosts will die as a result of the multiplication of only one bacterium (Meynell & Stocker 1957). This has two implications which can be tested. One is that for low doses the time to death will tend to become constant because it relies on the multiplication of only one organism (Meynell & Meynell 1958). The other is that if a mixture of variants of equal

virulence of a microbe is given, at low doses only one variant should usually be found at post mortem (Meynell & Stocker 1957). Testing time to death at low doses proved difficult: few hosts died so the confidence intervals were wide. The results, using salmonellae in mice, were inconclusive, both from Meynell's and more recent experiments (Meynell & Meynell 1958, Hormaeche 1975).

Experiments using mixtures of variants of salmonellae in mice have shown that as the dose decreased the composition of the post mortem samples varied more in composition from mouse to mouse, with a few mice at low doses giving pure or nearly pure cultures of any one variant (Meynell & Stocker 1957, Meynell 1957). That fewer hosts than predicted showed pure cultures of a single variant was explained on the basis of a terminal breakdown in host resistance. Single variant blood cultures following mixed inocula have more recently been demonstrated in experiments using rats infected with *E coli* (Pluschke et al 1983), *H influenzae* (Moxon & Murphy 1978, Rubin & Moxon 1984) and a Group B streptococcus (Rubin 1987).

Further evidence in support of the hypothesis of independent action comes from experiments with respiratory infections of mice altering the time over which the inoculum was given. Using *Streptococcus zooepidermicus* and *Klebsiella pneumoniae* the proportion of mice dying was similar for each organism when the same dose was given as a single challenge or was divided and given as small daily doses over one month (Goldberg et al 1954). If co-operation between organisms were important the single challenge would be expected to be more effective.

The strongest evidence against the simpler stochastic models (1 and 2 above), but compatible with birth-death models, comes from viable counts carried out during experiments with intraperitoneal inoculations of salmonellae in mice. It was shown that multiplication of the whole inoculum occurred and that the growth of the whole inoculum determined the latent period. In mice given doses which they were likely to survive, there was initial multiplication of the whole dose before a fall in the counts (Meynell & Meynell 1958, Armitage et al 1965).

The basic birth-death model is unsatisfactory because of inconsistencies between its predictions and the experimental evidence (Armitage et al 1965, Meynell & Maw 1968) and because it predicts that if inoculated organisms succeed in increasing even slightly they are virtually certain to increase to an infinite extent (Williams & Meynell 1967). The model can be modified - for example, by allowing the birth and death probabilities to change with time or with the number of organisms (Williams & Meynell 1967). Viable counts on the lungs of mice

infected by intranasal inoculation of *Bordetella pertussis* suggested that growth rates were faster after small inocula (Dolby et al 1961). Maw and Meynell (1968) used a non-replicating phage in *S typhimurium* to calculate the number of bacterial divisions which had taken place after intravenous inoculation into mice. The multiplication rate was faster in the first hour than in the subsequent 2 days. By comparing bacterial generations with the individual colony counts they showed that both birth (division) and death rates varied between mice.

None of the models fit the data perfectly, but the points of interest here are the implications of the basic models for the relationship between dose and severity, and in particular, whether any of the models preclude a dose-severity relationship. If there is "co-operation" between microbes then a dose-severity effect seems likely. The challenge to the hypothesis of a dose-severity effect comes if independent action is assumed, with the implication that infections with low doses are often due to the progeny of a single organism. (Doses less than the ID50 may very well be those frequently encountered in natural infections (Blaser & Newman 1982).) Dose could still influence severity within this range of low doses, if the single organism is likely to establish itself and start to multiply earlier following a larger dose. This is discussed further below. First I shall consider the assumption of the critical load which is common to all models.

The problems of critical loads and end-points

The different models all describe the multiplication of the inoculum, or part of the inoculum up to a certain critical level. The assumption of a critical load determining response is rarely challenged, although Meynell (1963) does suggest the possibility that the level may increase with time (Figure 4.1b). The models are generally constructed around a single end-point, be it response or death, but a "mortality threshold" can be defined at a level corresponding to a load at an arbitrary point above the "morbidity threshold" (Williams & Meynell 1967). This assumes not just that a certain load of microbes in the host is necessary to produce response, but that a larger load gives a more severe response.

Fairly constant terminal loads of bacteria have been found in experiments with rodents using *S typhimurium* (Meynell & Meynell 1958), *Staphylococcus aureus* (Smith et al 1960, Gorrill and McNeil 1963) and anthrax (Smith et al 1954). In experiments with intranasal infections of *Bordetella pertussis* in mice (Dolby et al 1961), the critical viable counts in the lungs at the time of death were higher when death was more prolongued. Following intracerebral inoculation the critical level in the brain decreased with time (Standfast & Dolby 1961).

It remains to be seen whether load correlates with lower levels of morbidity, or with death in less artificial situations. Certainly the evidence from malaria, discussed in Section 3.4, suggests a poor correlation between parasite load and severity. Malaria also provides evidence of the "critical level" for morbidity changing over time, in terms of the parasite load associated with fever increasing during the course of the illness (Boyd 1938), and of very different critical levels (or pyrogenic thresholds) in different individuals (Boyd 1938).

Another problem with the concept of critical loads is that the models tend to assume an all or nothing response. This is obviously appropriate when death is the outcome, but for lesser degrees of illness is at best an approximation to reality. Illness can usually be thought of as a continuum from asymptomatic to "cases" to death (Rose 1992). Definition of illness is to some extent arbitrary and yet we know for many diseases that dose determines the attack rate for the level of illness that happens to have been chosen; that is, the dose determines the proportion above a certain point on the continuum.

In experimental human infections with enteric organisms (eg McCullough & Eisele 1951a,c,d, Hornick et al 1970), increasing the dose increases the proportion of people with positive stool cultures and higher doses are required to increase the proportion with clinical disease. In this system two levels of response have been distinguished. One is "infection" and is identified by positive stool cultures. The other is "disease" and is identified by clinical symptoms or signs. The "attack rate" could be determined for either outcome, and both are dose-dependent. Is this equivalent to dose determining the severity of disease itself or is disease truly a different process from infection?

In any study some individuals with positive stool cultures (infection) are likely to have minor symptoms but fail to meet the variable criteria chosen to represent disease. Some cases will be difficult to define and it is worth considering to what extent the distinction is real. Classically, infection implies multiplication of the organisms (in the bowel in this case) and disease implies some pathogenic effect of the organisms on the host, usually associated with some degree of invasion. Yet presumably the two processes overlap in time, and a certain amount of invasion and pathogenic activity is required before signs and symptoms are perceived, so the distinction in practice is at the point of detection rather than at the level of a change in pathogenesis. Defining illness is difficult but, as we saw in the typhoid volunteer data (Section 2.5), the definition of illness used can have a critical role in determining whether or not a true dose-severity relationship is detected, or whether a spurious dose-severity relationship is produced

by misclassification.

The more the pathogenic process appears to be part of the same continuum - one process determining "disease" and more of the same determining severe disease - the more likely it would seem to be that load, and therefore perhaps dose, should determine severity. If different disease processes occur at different stages, for example if the next stage of a disease depends on a small proportion of the microbes moving elsewhere in the body, then the total body load and the initial inoculum size are less obviously relevant.

The animal experiments using mixed variants of bacteria give us some insight into the point at which the pathogenic process may change. Intranasal inoculation of infant rats with mixtures of streptomycin-sensitive and streptomycin-resistant strains of *Haemophilus influenzae* type b of equal virulence gave rise to nasopharyngeal colonisation, bacteraemia and meningitis (Moxon & Murphy 1978). The nasopharyngeal cultures were predominantly mixed, except with very small doses, but the blood cultures were usually of a pure strain even with high doses. Bacteraemia occurring within the first 6 hours was more often mixed, but this early bacteraemia often resolved spontaneously. Cerebrospinal fluid cultures were only positive in those with bacteraemia, and usually contained the same strain as the blood. Two thirds of those with mixed bacteraemias gave pure CSF cultures. It was suggested that, following initial nasopharyngeal colonisation, which could be accompanied by transient bacteraemia, a focus of infection was established by one bacterium which gave rise to the blood infection.

Mixed inoculation experiments with *E coli* in infant rats showed a similar pattern (Pluschke et al 1983). The rats were fed mixtures of a single dose of lactose-fermenting and non-lactose-fermenting strains of an enteroinvasive *E coli*. Most of the animals developed mixed infections in the gut. Infections in the mesenteric lymph-nodes were similar to those in the gut, and therefore usually mixed, but only pure cultures (of either strain) were obtained from the blood. Meningitis was only observed in animals with high levels of bacteraemia. Here, although meningitis was related to blood load, since bacteraemia seems to derive from a single bacterium (presumably after lymph-node invasion) a relationship between dose and the proportion with meningitis might not be expected.

In these rat experiments there was a clear distinction between colonization and disease, but for a disease such as cholera in which the pathogenic events take place in the gut, then a more continuous relationship might be expected. In contrast, in typhoid the processes of disease are

very different from those of the initial colonization; a close association between dose and severity is intuitively less likely, and no association was found when mortality was used as the outcome (Section 2.3). In the volunteer experiments, however, in which fever was used to define disease and peak fever was a measure of severity, a correlation with dose was found. A direct relationship with load is also less likely if the damage to the host relies principally on inflammatory or immune responses. The relationship with dose could also be complex, in that both very large and very small doses can alter the nature of the immune response and induce tolerance (Bretscher 1992).

A simple compartment model

It may be useful to think of the body as a series of compartments, starting at the exterior (which includes the gut) and working in. The probability of organisms reaching and multiplying in each compartment depends on the number of organisms in the compartment immediately preceding it in the series, how easily they can penetrate the compartment, and how readily they can multiply once there. To establish an infection, organisms usually have to multiply at their point of entry which is usually an exterior surface. For the next stage of the pathogenic process they may need to move to another compartment, such as the blood or lymph nodes. If all of the dose and its progeny, or a fixed proportion of it, moves to the next compartment, then the initial dose can be expected to influence what happens next. If movement to the next compartment is "difficult" such that very few organisms penetrate and manage to multiply, then the load in the first compartment will influence the chance of this happening, but the next stage of the infection will arise from the progeny of the few organisms which manage to reach and multiply in the second compartment.

In other words, if there is a bottle-neck in the system due to difficulty in entering a compartment and/or managing to multiply there, then only a few of the progeny of the original dose can be expected to make it. In the extreme case only one organism is likely to get through and succeed in multiplying for each successful episode of disease. The further progress of the disease will depend on the organisms on the far side of the bottle neck. Of course there may be further bottle-necks, but it is the position of the first one which should determine whether, in this simple model, the initial dose affects just the attack rate or may also influence severity. If the first bottle-neck occurs at a point in the pathogenic process before symptoms arise which define the disease, then the infecting dose will determine only the attack rate. If the first bottle-neck occurs later, after the pathogenic processes in the compartment which gave

rise to some symptoms then the infecting dose will influence both the attack rate and the probability of proceeding to the next stage which may be interpreted as severity.

In the rat experiments with *H influenzae* (Moxon & Murphy 1978) the first bottle-neck appears to operate between nasal colonization and bacteraemia. There may be a second bottle-neck between blood and CSF infection (most mixed infections in the blood gave pure cultures in the CSF) but the infecting dose would no longer be expected to have an influence. In the experiments with *E coli* (Pluschke et al 1983) the first bottle-neck appears between mesenteric lymph-node infection and bacteraemia. If symptoms were experienced at the stage of the lymph-node infection, then the infecting dose might be considered to influence severity of disease; with a larger dose there would be an increased probability of proceeding beyond this level of infection to bacteraemia.

According to this hypothesis, for infectious agents which multiply only in one compartment, such as *V cholerae*, infecting dose should be able to influence severity. This does not mean that it necessarily does, as there could be other limiting factors: for example, if all the toxin receptors were saturated, a further increase in bacterial load would have no effect. (Similarly, for intracellular parasites, such as viruses, saturation of available cells could in theory occur.)

A disease process involving more than one compartment may be subject to more sources of host variation: different factors could limit microbial multiplication in each compartment. Even if dose did influence severity it might be harder to detect.

The nature of bottle-necks and the role of time

My use of the term "bottle-neck" is not meant to imply a physical barrier to microbes - although this could be part of the mechanism. The bottle-neck refers not just to the passage of the microbe into the next compartment but its survival and multiplication. In this respect, the effectiveness of the bottle-neck could change over time, increasing as host immunity increased, or decreasing in the late stages of a terminal illness, as suggested by Meynell and Stocker (1957). With a larger dose there should be a greater chance of a microbe reaching and multiplying in the next compartment, purely stochastically. Perhaps more importantly, this chance event is likely to happen earlier following a large inoculum, which could increase the chances of the microbe's success in multiplying in the next compartment. Indeed a microbe arriving earlier may have the opportunity to multiply more, giving a larger load which may

have greater pathogenic effects itself or have a greater chance of transferring again into the next compartment for a further stage of the pathogenic process. Adding time and associated changes in immunity to the model therefore has the effect of allowing the infecting dose to influence events beyond the bottle-neck *even if the disease results from the multiplication of a single microbe.*

In general, organisms with faster doubling times have shorter incubation periods and will have multiplied more times before the onset of the immune response. If the incubation period is very short compared to the time taken for the immune response to start then the infection will already be well established before the immune response starts to take effect, at least in non-immunes, and the change in the effectiveness of bottle-necks may come too late to affect the disease. If the incubation period is longer than the immune response then the bottle-necks may already be fully effective before they are challenged. In these conditions the simpler model may be more appropriate.

How well does the model fit the facts?

For the infections which I have discussed in detail in the thesis, how well does the model fit the relationships with dose which have been observed?

The food poisoning salmonellae give rise to diseases with short incubation period in which the pathogenic process occurs essentially in one compartment with only local invasion. Further penetration is rare (Mims 1987). Dose should be able to influence severity, and there is some evidence that it does so (Section 2.3-4).

In contrast, typhoid has an incubation period of 1-3 weeks and involves several compartments. After entering the gut the organisms invade the intestinal mucosa, passing into the lymphatics and then into the blood, probably via the thoracic duct. They then multiply in several organs, notably the liver, before returning to the gut via the bile duct, where they invade and multiply in Peyer's patches. Although death is an uncontentious measure of severity it can occur in many different ways involving different organ systems: haemorrhage or perforation of the gut, central nervous system involvement, pneumonia, or haematological complications. There are numerous opportunities for bottle-necks in this system and it is perhaps not surprising that the initial dose appears to have little influence on the later course of the disease (Section 2.3).

In malaria there are other dimensions to consider: the magnitude of the multiplication that occurs, and the change in the form of the parasites in the body which influences the immune response. The models of infection were established primarily for bacterial infection assuming binary fission. In malaria one sporozoite in the liver gives rise to 2000-40000 merozoites, depending on species, (McGregor 1965) and each subsequent erythrocytic cycle increases the number by 16-24 times (Garnham 1988). The incubation period is long, but it is not until they emerge from the liver, after the first huge phase of multiplication that merozoites are available to trigger the immune response. Vivax malaria can be considered as a three compartment disease (prehepatic, hepatic and erythrocytic).

When rats or mice were inoculated with large numbers of sporozoites of *Plasmodium berghei*, there was a positive correlation with the number of extra-erythrocytic forms per mm^3 of liver tissue (Verhave 1975, Vanderberg 1977). In rats about 30% of inoculated sporozoites gave rise to extra-erythrocytic forms, and in mice about 4% (Vanderberg 1977). When mosquitos were used and each animal was bitten by 50 mosquitos there was a correlation between the mean intensity of salivary gland infection and the number of extra-erythrocytic forms (Vanderberg 1977). To demonstrate extra-erythrocytic forms in human volunteers, Shortt et al (1948, 1949) used very large inocula in order to ensure that extra-erythrocytic forms would be present in the small amount of tissue available by liver biopsy. These experiments with artificially high inocula suggest that, although not all the sporozoites gave rise to hepatic schizonts, the infecting dose influenced the number which did occur.

In experiments with human volunteers bitten by 5 mosquitos infected with *P falciparum*, Davis et al (1989) used the measured parasitaemia and prepatent period to calculate the number of infected hepatocytes and hence the number of sporozoites from which the infections originated. These calculations assumed that one sporozoite infected one liver cell giving rise to 30000 merozoites, each of which infected one erythrocyte giving rise to 16 merozoites every 48 hours. Among 4 volunteers the calculated number of infected hepatocytes ranged from 4 to over 300 with a mean of 120, suggesting an average inoculum of 24 sporozoites per mosquito. Direct *in vitro* measurements of sporozoite transmission suggest that the median number transmitted per mosquito is similar to this (Rosenberg et al 1990, Beier et al 1991 a & b, Ponnudurai et al 1991). If this is applicable *in vivo*, it suggests that there is very little loss in the predicted number of parasites before the blood stage is reached. In terms of the compartment model, there does not appear to be a significant bottle neck before the blood stage, and dose could therefore, theoretically, influence severity. Possible reasons why this was

not found were discussed previously (Section 3).

The lack of a demonstrable association does not imply that dose does not influence severity

This thesis has concentrated on methods of investigating whether dose influences severity and on trying some of them in practice. The only way to get a straightforward answer to the question would be to conduct experiments in genetically and environmentally identical animals. Such experiments would tell us whether, everything else being equal, dose could influence severity of disease. They would not however give us the answer to the far more important question of whether, given the natural variation in hosts and conditions, dose has an appreciable influence on severity, nor the relative importance of any such influence. Epidemiological methods provide the tools for answering the question in natural situations, but in practice are beset by biases, particularly misclassification in the measurement of dose, and by confounding when indirect measures are used. Induced infections in humans provide the best data but their scope in terms of different diseases, range of doses used, severity of symptoms allowed before treatment is started, and numbers of subjects is obviously limited.

Findings of no association between dose and severity in these studies is difficult to interpret as implying a true lack of association because much of the bias is towards underestimation of an association. However, when incubation period is used as marker of dose, confounding by susceptibility will tend to overestimate an association.

It is unlikely that there is a single answer to the question of whether dose influences severity. It may be that dose influences severity in all diseases (except those where one microbe is uniformly fatal) and that it is only the relative importance of this influence which varies in relation to all the other causes of variation between individuals. The conclusions that can be reached from the diseases which have been studied in detail are that a strong dose-severity effect is unlikely in typhoid but may occur in food-poisoning salmonellae, and that if there is a dose-severity relationship in malaria, it is less strong than is generally supposed.

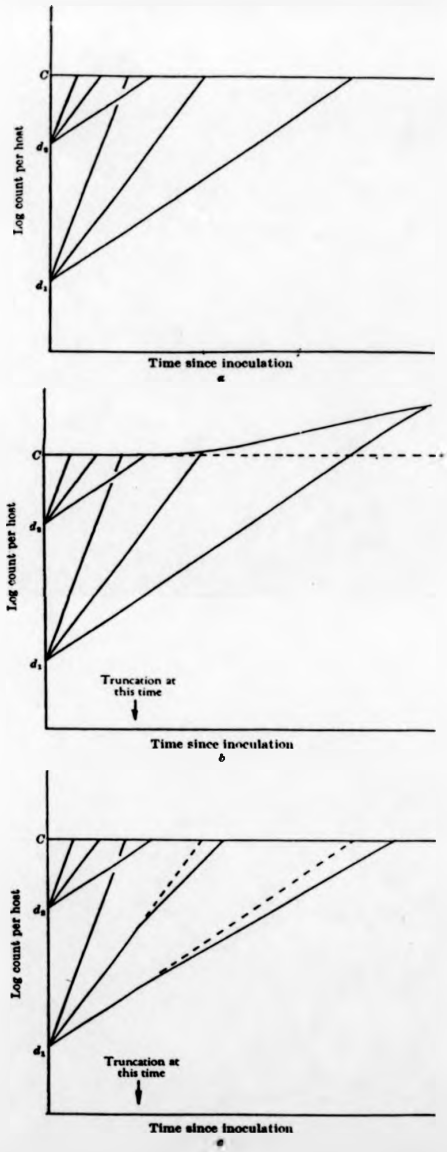


Figure 4.1. Hypothetical growth curves from infected hosts given doses d_1 and d_2. The curve for the mean rate in each group is shown between the curves for rates 0.5 and 2.0 times the mean. C is the critical level for response. (a) Constant multiplication rates and critical level over time. (b) Increase in critical level with time. (c) Fall in rate of increase of organsisms over time. (From Meynell 1963.)

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APPENDICES

APPENDIX 1. EPIDEMICS USED IN THE ANALYSIS OF TYPHOID EPIDEMICS (SECTION 2.3)

Reference	Year	Place	Vehicle	Median incubation period (days)	Attack rate		Case fatality rate	
					%	No.	%	No.
Scott 1934	1881	Blackburn	Water		0.61	?/720000	13.19	24/182
Stallybrass 1931	1885	Pennsylvania	Water		12.55	1004/8000	11.35	114/1004
Scott 1934	1893	Worthing	Water		8.33	1298/15579	12.48	162/1298
Scott 1934	1893	Worthing	Water		3.87	113/2919	21.24	24/113
Greenwood 1935	1897	Maidstone	Water		8.08	?/720000	6.81	132/1938
Bulstrode 1902	1902	Winchester	Oysters[1]	14	8.20	10/122	40.00	4/10
Bulstrode 1902	1902	Southampton	Oysters[1]	16	9.17	10/109	10.00	1/10
Ledingham 1910	1904	Brislington	?Carrier[1]		72.22	26/36	7.69	2/26
Scott 1934	1904	Lincoln	Water		1.95	1058/54204	11.81	125/1058
Bracken et al 1911	1908	Minnesota	Water	15	4.40	440/10000	6.82	30/440
Johnstone 1910	1909	Conway	Milk[1]		11.11	26/234	18.18	10/55
Carnseth 1912	1910	Oakenshaw	Milk[1]		11.78	53/450	7.94	5/63
Grover 1912	1911	Iowa	Water		2.83	170/6000	10.00	17.170
Wheaton 1913	1912	Stood, Kent	Water		0.51	69/13428	11.59	8/69
Macewen 1912	1912	Ringwood, Hants	Water[1]		50.00	23/46	17.39	4/23
Hutchinson 1913	1912	Colne, Lanca	Milk[1]		7.44	67/900	8.57	6/70
Jordan & Irons 1912	1912	Rockford, Ill	Water	20	0.42	199/47500	12.06	24/199
Lumsden 1912	1912	Iowa	Water	14	0.71	11/1550	27.27	3/11
Ridlon 1912	1912	Texas	Milk[1]		1.26	25/1950	2.94	1/34
Manby 1914	1913	Kenilworth	Water		0.84	44/5256	9.09	4/44
Jordan & Irons 1913	1913	Quincy, Ill	Water	26	0.55	202/37000	7.92	16/202
Sawyer 1914	1914	Hanford, Ca	Spaghetti[1]	7	56.67	85/150	3.23	3/93
Geiger 1917	1915	Colusa, Ca	Milk[1]	12	3.58	23/643	0.00	0/23
Cumming 1917	1916	Helm, Ca	Icecream[1]	6	95.93	23/24	13.04	3/23
Geiger et al 1917	1917	California	Water		20.00	52/260	0.00	0/52
Anon 1921	1920	Salem, Ohio	Water		7.85	785/10000	1.53	12/785
Shaw 1922	1921	Bolton-Deane	Water		0.67	137/20497	11.68	16/137
Shaw 1922	1921	Bolton-Deane	Water		7.26	260/3581	11.15	29/260
Bundesen 1925	1924	Chicago	Oysters	12			12.40	16/129
Lumsden 1925	1924	Tennessee	Milk[1]		33.33	100/300	8.00	8/100
Ramsay 1926	1925	Michigan	Food[1]	13	14.00	35/250	17.14	6/35
Murata 1928	1925	Japan	?Food[1]	18	48.68	37/76	20.45	9/44
Stallybrass 1931	1926	Hanover	Water		0.59	2500/425000	10.40	260/2500
Stallybrass 1931	1927	Montreal	Milk		5.10	3601/70576	10.66	533/5002

Reference	Year	Place	Vehicle	Median incubation period (days)	Attack rate		Case fatality rate	
					%	No.	%	No.
Anon 1928	1928	Royal Navy	Lettuce[1]	12[2]	10.22	95/930	7.37	7/95
Dean 1931	1928	New York State	Water		1.15	248/21599	10.08	25/248
Lessa 1930	1929	Rio de Janeiro	Water[1]		18.31	39/213	12.82	5/39
Miner & Forsbeck 1929	1929	Massachusetts	Chicken[1]	19	44.62	29/65	6.90	2/29
Garrido-Morales & Costa Mandry 1931	1931	Porto Rico	Water[1]		33.33	10/30	10.00	1/10
Anon 1932	1932	Derby Dale	Water		5.07	71/1400	8.45	6/71
Magliano 1935	1932	Genoa	Water		2.51	42/1672	7.14	3/42
Gomez Jimenez 1934	1932	N Spain	Water		7.79	87/1117	4.80	4/87
Leader 1932	1932	Massachusetts	Milk[1]		39.68	25/63	7.14	2/28
Ritchie & Armstrong 1932	1932	Dumfriesshire	Water		5.50		3.13	2/64
Shaw 1933	1933	Malton, Yorks	Water		5.22	235/4500	8.52	23/270
Morris 1935	1933	New South Wales	Food[1]		11.00	33/300	27.27	9/33
Hornung 1934	1934	Black Forest	Water[1]	17			9.09	3/33
Anon 1935	1935	Lourdes	?		6.82	75/1100	5.33	4/75
Johnson 1936	1935	Philadelphia	Salad[1]		33.77	77/228	6.98	6/86
Anon 1936	1936	England	?[1]		35.00	14/40	14.26	2/14
Watson Smith 1942	1936	Bournemouth	Milk	14[3]	5.18	518/10000	9.85	51/518
Roelcke 1937	1936	Mitelbadan	Icecream[1]	13			0.00	0/24
Lembacke & von Haesseler 1936	1936	Massachusetts	Salad[1]	9	37.14	13/35	7.69	1/13
Holden 1939	1937	Croydon	Water		0.75	?/142000	13.87	43/310
Caudill 1937	1937	Kentucky	Water[1]		22.86	16/70	12.50	2/16
Landau 1938	1938	S Africa	Milk[1]		30.06	52/173	20.90	14/67
Old & Gill 1940	1939	Louisiana	Oysters[1]	9	75.00	87/116	9.20	8/87
Sirois 1942	1942	Canada	Milk[1]		27.50	66/240	10.29	7/68
Constam & Steiner 1945	1943	Switzerland	Water[1]		33.75	27/80	3.70	1/27
Galea 1944	1943	Malta	Water		0.99	1275/128788	12.20	156/1275
Jordan & Evarley Jones 1945	1944	Middle East	?Food[1]		34.35	79/230	11.25	9/80
Marmion et al 1953	1950	Egypt RAF Unit	Mock cream	7[3]	34.06	234/687	0.0	0/234
Neill et al 1958	1958	Monark	Water	21	6.18	34/550	2.94	1/34
Caraway & Bruce 1961	1961	Louisiana	Chicken	20	32.00	31/97	0.00	0/31
Bernard 1966	1963	Zermatt	Water	17	4.37	437/10000	0.68	3/437
Mallory et al 1968	1968	Audrain, USA	Water	18[4]	23.36	25/107	0.00	0/25
Taylor et al 1974	1971	Trinidad	Icecream	19	1.20		0.00	
Hanson et al 1972	1971	Pennsylvannia	?Food	17	25.36	33/130	6.06	2/33

[1] Circumscribed pre-1945 outbreaks (see text)
[2] Modal incubation period
[3] "Average" incubation period

APPENDIX 2. SALMONELLA EPIDEMICS FROM CDC SALMONELLA SURVEILLANCE (SEE SECTION 2.3)

(References give issue and page no. Incubation period is median, in hours)

Ref	Place	Date	Vehicle	AR % (No.)		HR % (No.)		Incubation
S enteritidis								
38:5	New Jersey	1965	?	50.0	(65/130)	4.6	(3)	
77:4	Ohio	1968	Icecream	100.0	(12/12)	8.3	(1)	
93:2	Alaska	1969	Whale	95.9	(93/97)			9[1]
99:5	Columbia	1970	Picnic	48.1	(181/376)	1.1	(2)	28[1]
101:4	Pennsylvania	1970	Salad		(130/ .)	23.1	(30)	18
102:2	Florida	1970	? Turkey	15.4	(139/900)	14.4	(2)	
103:2	Michigan	1970	Prison cafe	40.3	(353/876)	6.2	(22)	
104:2	Georgia	1970	Icecream	92.3	(12/13)	100.0	(12)	
104:2	Nebraska	1970	Roast meat	12.6	(252/2000)	6.8	(17)	
116:2	California	1972	Ham ?	41.0	(41/100)	0.0	(0)	
116:2	Rhode Island	1972		5.8	(10/172)	20.0	(2)	
117:2	Oregon	1972		12.9	(17/132)	5.9	(1)	
123:2	Indiana	1974	Icecream	25.0	(25/100)	32.0	(8)	
S infantis								
71:5	Kentucky	1968	Ham	67.3	(37/55)	27.0	(10)	20
85:2	Tennessee	1969	Smoked turkey	64.7	(11/17)	18.2	(2)	29[1]
85.3	Texas	1969	Turkey	53.9	(28/52)	14.3	(4)	11
116:3	Kansas	1972	Icecream	100.0	(12/12)	91.7	(11)	
117:2	Illinois	1972	Bread dressing	12.7	(38/300)	26.3	(10)	
120.2	Oregon	1973	Roast beef	27.3	(123/450)	0.0	(0)	
123:2	Texas	1974		12.5	(50/400)	6.0	(3)	
S thompson								
44:3	St Louis	1965	Icecream	92.3	(12/13)			18
95:2	New Orleans	1969	Church supper	99.5	(200/201)	25.4	(18)[3]	13[1]
99:1	Tennessee	1970	Barbecued pork		(303/)	17.8	(54)	54
102.2	New Jersey	1970		65.0	(130/200)	20.0	(26)	
112:2	Maine	1971	Chicken salad	51.5	(17/33)	0.0	(0)	29[1]
113:2	Iowa	1971	Restaurant	43.2	(95/220)	23.2	(22)	
116:3	Pennsylvania	1972	Coconut cream	3.1	(31/1000)	38.7	(12)	
119:2	Florida	1973	Inflight food	14.8	(17/115)	11.8	(2)	
120:2	Los Angeles	1973	Custard pie	100.0	(23/23)	0.0	(0)	
120:2	Pennsylvania	1973	Roast beef	80.7	(25/31)	12.0	(3)	

Ref	Place	Date	Vehicle	AR % (No.)		HR % (No.)		Incubation
S typhimurium								
30:8	New Jersey	1964	Wedding	14.9	(35/235)			15
33:4	Michigan	1964	Icecream	81.8	(9/11)			19
38:2	California	1965	Water	12.7	(14000/110000)	0.5	(75)	
43:7	N Carolina	1965	Potato salad	50.0	(244/488)	4.1	(10)	96
59:4	Montana	1966	Turkey	54.4	(31/57)	6.5	(2)	48[2]
63:5	Tennessee	1967	Potato salad	35.7	(215/602)			32
77:2	N Carolina	1968	Icecream	89.5	(17/19)	0.0	(0)	
77:4	New Jersey	1968	Cafe	42.0	(245/583)	0.0	(0)	21[1]
83:2	New York	1969	Spaghetti	50.0	(13/26)	15.4	(2)	34[1]
102:2	N Carolina	1970	Barbecued ham	74.7	(56/75)	32.1	(18)	
104:2	Missouri	1970	Icecream	100.0	(11/11)	0.0	(0)	
105:1[4]	Tennessee	1970	Barbecued pork	95.2	(40/42)	10.0	(4)	24[1]
105:1[4]	Tennessee	1970	Barbecue	70.6	(12/17)	8.3	(1)	30
105:1[4]	Tennessee	1970	Turkey	87.3	(144/165)	2.8	(4)	
110:2	Michigan	1971	Smoked fish	75.7	(28/37)	10.7	(3)	
112:2	New Jersey	1971	Roast beef	61.1	(22/36)	27.3	(6)	
115:2	New Jersey	1972	Bakery cakes	12.5	(150/1200)	2.0	(3)	
118:2	Wisconsin	1972	Hamburger ?	26.7	(20/75)	10.0	(2)	
116:3	Virginia	1972	Icecream	60.0	(45/75)	6.7	(3)	

[1] Incubation is mean
[2] Incubation is "average"
[3] Information for 71 cases only
[4] Same restaurant

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