SEROEPIDEMIOLOGY OF RUBELLA VIRUS IN ENGLAND AND THE DESIGN OF CONTROL PROGRAMMES BASED ON ~~MASS~~ VACCINATION

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ABSTRACT

Epidemiological data for rubella has been acquired from the serological analysis of horizontal cross-sectional surveys of South East England (1980-84) and South Yorkshire (1969, 73, 76, 79, 81, & 85).

The radial haemolysis test, modifidied to produce standardised quantitative measurements, was selected to screen serum samples (in excess of 6000), from the two study populations, for rubella-specific antibodies.

The distribution of immunity to rubella in the population has been described and an assessment made of the influence of the current policy of immunization. Elevated levels of seropositivity in adolescent girls and women of childbearing age as a result of selective immunization are recorded. General levels of herd immunity remain largely unaltered from the pre-vaccination period.

Analysis of age-serological profiles for both study populations using a 'polynomial catalytic infection model' revealed age-related changes in rubella transmission rates. The force of infection varies from low in young children to high in 5-15 year olds to low again in adults.

Similar analyses have been applied to available rubella case notifications. Findings from the two sources of empirical data are compared, with emphasis placed on the accuracy of estimated age-specific rates of transmission.

Mean rubella antibody concentrations are shown to decline with increasing age, accompanied by an increase in antibody concentration variability. There is evidence to suggest that population levels of antibody in vaccinated female age classes are significantly lower than in unvaccinated males of the same age.

Strong association between specific HLA-DR alleles and rubella antibody concentration has been uncovered in a sample of 152 unrelated juvenile chronic arthritis patients. The importance of genetic factors to humoral immunity in the general population could not be established from analyses of normal healthy adults.

Various alternative rubella immunization policies for the United Kingdom have been evaluated using a mathematical model of rubella virus transmission. Attention is focussed on the robustness of model predictions of congenital rubella incidence to parameters estimated from the accumulated database of epidemiolgical information and on the importance of future trends in vaccine uptake and age-specific fertility.

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TEXT ABBREVIATIONS

APP	Age-prevalence profile
ASP	Age-serological profile
BTC	Blood Transfusion Centre
CDC	Center for Disease Control
CDSC	Communicable Disease Surveillance Centre
CFB	Complement fixation buffer
CRS	Congenital rubella syndrome
DHSS	Department of Health and Social Security
ELISA	Enzyme-linked immunosorbent assay
EUC	European caucasians
HA	Haemagglutinating antigen
HI	Haemagglutination inhibition
iu	International units
JCA	Juvenile chronic arthritis
NCRSP	National Congenital Rubella Surveillance programme
OPCS	Office of Population Censuses and Surveys
PHLS	Public Health Laboratory Service
RCGP	Royal College of General Practitioners
RH	Radial haemolysis
SRBC	Sheep red blood cells
WAIFW	´Who Acquires Infection From Whom´

Chapter 1

GENERAL INTRODUCTION

1.1 Disease Associated with Rubella Virus Infection

Rubella is a common acute viral infection which occurs predominantly during childhood and adolescence (Herrmann, 1979; Lamprecht, 1984; Banatvala & Best, 1984). When acquired postnatally, rubella usually causes a mild self-limiting illness. The characteristic symptoms of low-grade fever, lymphadenopathy and exanthematous rash may often go unnoticed. Indeed, wholly inapparent infections, proven by serological methods (Brody, 1966) are thought to be common, particularly in children (Green et al, 1965; Krugman et al, 1953). Other features of the disease, such as joint involvement (most common in adults), occur less frequently and the severe complications of thrombocytopaenic purpura and enchephalitis are very rare (Lamprecht, 1984).

Following its recognition as a specific entity by German physicians in the early 1800's (hence the name German measles) and until relatively recent times, rubella was generally regarded as the most benign of common childhood viral or bacterial infections. Interest in it lay mostly as a differential diagnosis from measles and

scarlet fever.

This misguided image of rubella as an infection of little consequence is substantially altered today as a result of evidence (in 1941) which associated maternal rubella with congenital abnormalities of the unborn offspring (Gregg, 1941). Gregg observed congenital cataracts in a high proportion of infants whose mothers had been infected early in pregnancy during an unusually large epidemic of rubella in Australia in 1940. These babies, it was noted, were also of low birth weight, and over half were suffering from defects of the heart.

Identification of more cases in the ensuing years confirmed the teratogenic effect resulting from transplacental transmission of the rubella virus (Wesselhoeft, 1947). During this time nerve deafness and mental retardation were recognised as other frequent defects associated with prenatal rubella. Subsequently, in the diagnosis of congenital rubella, a classic triad of the most commonly observed specific abnormalities, namely, cataracts, heart defects and deafness, was established.

The severe epidemics of 1962-65 in Europe and N. America brought to light many other rubella-related congenital defects, particularly those signalling ongoing chronic viral infection (Krugman (ed.), 1965). These added to the existing array of abnormalities which was then collectively termed the congenital rubella syndrome or CRS (see Hanshaw & Dudgeon, 1978; Sidle, 1985, for reviews of congenital rubella).

The widespread epidemics in the early sixties were also

instructive in revealing the magnitude of the congenital rubella problem in developed countries. In the USA alone, the epidemic of 1964 resulted in an estimated 20,000 CRS cases and 30,000 stillbirths (CDC, 1969a). Morbidity and mortality of such proportions, as well as the vast expense incurred by governments for health care, clearly indicated the need for control measures to prevent intrauterine rubella infection.

1.2 Prevention of Congenital Rubella Infection

The underlying principle for the control of infectious diseases is the interuption of transmission between the source of contamination and the susceptible host. Based on this premiss there are, essentially, only three methods by which the spread of infection can be checked:

(i) Elimination of the contaminative source;
(ii) Reduction in the frequency of contacts between susceptibles and the source of infection;
(iii) Removal of individuals from the susceptible class directly into the immune class, as with vaccination.

Which of these approaches is adopted will be dependent upon the nature of the causative agent and the mode of transmission.

For viruses, particularly those of childhood, which are contracted directly via respiratory secretions from

infectious persons, immunization is perhaps the only practical option for the control of infection. The absence of effective anti-viral drugs limits the use of therapy in reducing the principal source of contamination, i.e. the infectious individual. Quarantine of persons infected with, for example, measles or mumps, has proved unsatisfactory as a means of curtailing the contact rate between susceptible and infectious individuals in a community (due to the period of time prior to development of symptoms during which virus may be shed). By contrast, any disease associated with an antigenically stable virion and long-lasting immunity to re-infection lends itself to control by vaccination (see Banatvala & Best, 1984, for details pertaining to rubella).

It is therefore not surprising that for the prevention of congenital rubella infection, attention was focussed on the development of a vaccine (see report of International Conference of Rubella Immunization in 1969, Krugman (ed.)). This became a realistic objective following isolation of the aetiologic agent by in vitro culture in 1962 (Weller & Neva, 1962; Parkman, Buescher & Artenstein, 1962). At this time there were also encouraging signs of success from programmes of immunization against pertussis, poliomyelitis and smallpox (Evans (ed.), 1982; Anderson & May, 1982a).

In 1969 three live attenuated vaccines were licensed for marketing (Krugman (ed.), 1969). Since then programmes of mass immunization have been introduced into most of North America and Europe, also countries such as Australia, Japan, India, Israel and China (see Burke, Hinman & Krugman (eds.), for review of current rubella vaccination programmes

worldwide). The primary objective of all rubella immunization policies is the prevention of infection in pregnant women, and thus protection of the unborn offspring from congenital rubella. Although rubella immunization strategy varies from country to country there are, broadly speaking, two approaches to the problem which are clearly illustrated by the policies of the United Kingdom and the United States of America.

The mainstay of the rubella control programme in the USA has been the mass vaccination of pre-school children (males and females) (CDC, 1969b). From the onset in 1969, it was reasoned that childhood immunization would not only directly protect females when they became fertile, but by reducing the dissemination of virus from where it was greatest, that is, in children, the risk of exposure to rubella infection in the community could also be reduced or perhaps eliminated. This would provide indirect protection of susceptible pregnant women (Bart et al, 1985a; Hinman et al, 1983, Krugman (ed.), 1969).

Rubella vaccine was rapidly accepted by the US population and a very high coverage has been achieved (Bart et al, 1985b). This is a consequence of using combined measles, mumps and rubella vaccine and of making rubella vaccination a requirement for kindergarten entry. As a result, the incidence of rubella and congenital rubella has declined to record low levels (CDC, 1986) and the total eradication of CRS seems a real prospect for the near future.

In the UK a selective programme of immunization is in

operation. Vaccine is administered to a large proportion of schoolgirls between the ages of 10 and 15 years (todays levels are in excess of 85% by the age of 15; DHSS statistics), and, in addition, is offered to adult women of childbearing age found to be serologically negative (in particular, to those immediately post-partum and those with an occupational risk of infection) (JCVI, 1982). The intention of such a policy is to reduce the proportion of susceptible females in the 'at-risk' years of fertility (and subsequently reduce the potential number of CRS cases) without, however, abrogating the circulation of wild virus in the community. Indeed, the pattern of rubella incidence has remained unchanged since initiation of the policy in 1970 (Tobin et al, 1985). Thus rubella infection has been retained as the major mediator of immunity in the population, whilst adolescent and adult female immunization supplements immunity levels in the critical childbearing years.

The selective immunization campaign in the UK was chosen, in preference to a programme of childhood immunization, to compensate for uncertainty over the duration and solidity of vaccine-induced immunity (Sidle, 1985; Dudgeon, 1985; Krugman (ed.), 1969). At the time it was recognised that this approach would cause only a gradual decline in the incidence of congenital rubella infection, with the full benefit deferred until the 1990's. Surveillance of the annual number of CRS cases in England and Wales by the National Congenital Rubella Surveillance Programme (Smithells et al, 1985) and of rubella associated

therapeutic terminations of pregnancy (OPCS series AB, 1986), from 1970 to the present, confirm these initial expectations of the policy (see Fig. 6.1).

Undoubtedly the success of rubella vaccination in this country, although significant, has been less dramatic than in the USA. Consequently there is some disatisfaction with the current UK policy (Brook, 1983; CDSC, 1987; Miller et al, 1985) and debate is now in progress to decide how best to make improvements (Dudgeon, 1983; Miller & Miller, 1985; Anderson & Grenfell, 1986b; Report of meeting held at CDSC, London in 1986). The issue of contention is whether to enhance vaccine uptake by teenage girls and adult women alone, or to include immunization of pre-school girls and boys to form a combined UK and USA strategy.

1.3 The Requirement for Rubella Seroepidemiological Information

Granted that there is some discontent with the current rubella immunization policy in this country, there is a need to feel certain that the outcome of any changes decided upon are likely to be beneficial. Any community-based immunization campaign is an intervention into a complex dynamic system of interaction between the host population and infectious agent (Fine & Clarkson, 1983). Therefore the consequences of imposing a programme of vaccination on a population may not be obvious and, hence, could be difficult to predict.

For instance, epidemiological studies have shown that mass childhood immunization (against, for example, measles, mumps, pertussis and rubella) acts to reduce the number of infectious individuals (and subsequently lowers the incidence of infection) but has little effect on the total number of susceptibles in the community until the infection is on the verge of eradication (Fine & Clarkson, 1982b, 1984; Anderson & May, 1982a, 1983). To many this is a surprising outcome and a sound understanding of the herd immunity effects of vaccination (Anderson & May, 1983, 1985a) is required to comprehend it. Observations of this sort indicate that there should not be a total reliance upon intuitive reasoning in the design of immunization strategies.

In order to make more objective decisions on the choice of vaccination policy, mathematical models of infectious disease transmission have been developed (Knox, 1980; Dietz, 1981; Hethcote, 1983; Anderson & May, 1983). Unfortunately, predictions based on such models, are liable to error if the assumptions upon which they rest are invalid or fail to adequately reflect the true epidemiological characteristics of the infectious agent (Fine & Clarkson, 1983; Anderson & May, 1983, 1985a).

Recent research has highlighted two features of the transmission dynamics of childhood viral and bacterial infections, and in particular of rubella, which are of importance in this respect. These are, the pattern of age-related changes in the rate of transmission, and variation in the degree of mixing of individuals within and

between age classes (Anderson & May, 1983, 1985a; Schenzle, 1984; Grenfell & Anderson, 1985). Refinements in the structure of the latest mathematical models take these two details into account, thus providing a better representation of the behaviour of infection in the host population (Anderson & May, 1985; Anderson & Grenfell, 1986).

However, the use of these models and the subsequent precision of their predictions remains dependent upon the existence and accuracy of empirical epidemiological data. Such information is required for two reasons; (i) in order to estimate model parameters, for example the age-specific rates of infection, and (ii) to enable comparisons to be made between model predictions and observed events.

Thus there is a need to accumulate relevant empirical data, of which seroepidemiological information forms an important aspect (Anderson & May, 1983, 1985a; Anderson & Grenfell, 1986). It is somewhat puzzling, therefore, that despite the availability of well developed serological techniques for the determination of rubella antibody status (which has resulted from the major commitment of virus laboratories to rubella diagnosis and screening (Morgan-Capner, 1984)) and an established requirement to monitor the impact of immunization programmes (Noah, 1980), there is a dearth of detailed seroepidemiological information pertaining to rubella in this country (Nokes, Anderson & Anderson, 1986). It was an awareness of this situation which led to the initiation of the present study, the aims of which are described in the next section.

1.4 Aims of the Present Investigation

The following is a list of aims of this project which were formulated prior to its initiation and those which became apparent as the investigation progressed:

(i) Create a large database of serological information on the distribution of immunity to rubella in this country, by employing a well evaluated serological screening technique on readily available serum specimens from individuals over a wide range of ages and of both sexes.

(ii) Modify and standardize the chosen serological test to facilitate the estimation of rubella antibody concentration in each serum sample during the routine screening process.

(iii) Analyse the epidemiological data obtained to (a) assess the impact of rubella immunization in the UK on the proportion of seronegative women of childbearing age, (b) estimate the indirect effects of this immunization on herd immunity and (c) establish the nature of age-dependence in the rate of transmission of rubella in the community.

(iv) Compare the information acquired from serology with that obtained from available rubella case notifications and determine which of the two is the most reliable for use in epidemiological research, particularly in the estimation of age-related transmission rates.

(v) Use the quantitative serological data to (a) determine the relevance of age-related trends in mean rubella antibody concentration in the population to the duration of natural or vaccine-induced rubella immunity and (b) reveal any association between rubella antibody concentration and specific genetic markers.

(vi) Use a mathematical model of rubella virus transmission (Anderson & May, 1985a) to (a) evaluate the impact of a number of future rubella immunization policies for the United Kingdom, (b) assess the robustness of model predictions to parameters estimated from various sources and (c) evaluate the importance of current trends in age-specific fertility and vaccine uptake to the choice of vaccination policy.

Chapter 2

SEROLOGICAL METHODS

2.1 Introduction

Screening for evidence of past rubella infection may be performed using a number of serological techniques which detect rubella-specific IgG. These include the haemagglutination inhibition (HI) test, the enzyme-linked immunosorbent assay (ELISA), and the radial haemolysis (RH) technique. The test of preference for a seroepidemiological survey should fulfill a number of criteria apart from specificity to the antibody raised against the virus in question. For example, (i) an appropriate sensitivity such that low seropositives are rarely mistaken as seronegative, (ii) rapid and easy operation to facilitate the screening of large numbers of samples, (iii) a high degree of reproducibility of results to enable comparison with other surveys and to authenticate conclusions derived from statistical analysis, and (iv) economical to run.

In the UK the radial haemolysis, or RH, test has become established as the major assay for the determination of rubella immunity both in diagnostic laboratories and in serological surveys (Pattison (ed.), 1982; Clarke et al,

1983; Miller et al, 1985). It is a test which is ideally suited to the measurement of rubella antibody concentration in large numbers of serum samples, that satisfies the criteria outlined above and, as a consequence, has been utilized in this present seroepidemiological investigation.

The basis of the RH test is the specific lysis of erythrocytes sensitized by haemagglutinating antigen (HA). Serum to be tested is allowed to diffuse radially from wells cut into an agarose gel in which are suspended the antigen-coated red blood cells together with complement. Specific antibody, if present in the serum, combines with the antigen to form an antigen-antibody complex that binds complement, and results in haemolysis in the form of a clear zone visible to the naked eye and easily measured. Anti-viral IgG (except early onset IgG) is the major mediator of the process; IgM is not detected (Neumann & Weber, 1983) (this represents an inability of the RH test to positively identify serum from individuals in the early stages of an infection, but cannot be regarded as an important deficiency for serological surveillance since the proportion of the population falling into this category at any one time will be very small).

The RH technique has previously been applied to a number of haemagglutinating viruses, such as influenza (Russel, McCahon & Beare, 1975) and mumps (Grillner & Blomberg, 1976). However, it has proved particularly useful in the detection of antibody to rubella virus (Skaug, Orstavik & Ulstrup, 1975; Pattison (ed.), 1982).

Evaluations of the rubella RH test have found it to be simple to perform, inexpensive, with good reproducibility (of results) and able to detect low levels of specific antibody (Clarke et al, 1977; Forger & Gilfillan, 1979; Kurtz et al, 1980; Neumann & Weber, 1983). In addition, the diameter of haemolysis zones is in proportion to the concentration of antibody present in the test sera, and, therefore, the results produced are of a quantitative nature.

The RH test provides a number of advantages over the main alternative rubella-IgG screening techniques. Unlike the HI test, RH is unaffected by non-specific inhibitors of haemagglutination (and as a consequence it offers a superior degree of sensitivity), furthermore, the RH test is less labour intensive and less expensive than either HI or ELISA (Champsaur, Dussaix & Tournier, 1980; Enders & Knotek, 1985).

In the present study over 7000 serum samples obtained from various sources in England (see Tables 3.1, 5.2 & 5.3) have been screened quantitatively for rubella-specific IgG using, in essence, the standard Public Health Laboratory Service (PHLS) RH technique (Kurtz et al, 1980) employed by virology laboratories across the country. Attention has been focussed on the standardization and reproducibility of quantitative antibody measurements. This has necessitated a number of modifications to the basic test giving rise to two methods, referred to as Methods I and II, which incorporate the use of standard sera of known potency for the determination of antibody levels of test sera, and the

monitoring of inter-test variability. Each method was used to screen a distinct set of serum samples and both are described below.

Two further tests, the HI and a commercial ELISA (Rubazyme, Abbott Diagnostics, UK), were employed as backup tests to confirm equivocal RH results. Although differences in sensitivity and in the type of antibody detected exist between the tests, comparisons have shown all three to yield highly correlated results (Neumann & Weber, 1983; Grillner, Forsgren & Nordenfelt, 1985).

This chapter deals only with the serological methodology, details of the analytical techniques and of the serum samples comprising the surveys reported in this project are given at the appropriate point in subsequent chapters.

2.2 Methods and Materials

(i) Radial haemolysis test - Method I

This method was used to quantitatively screen serum samples from the surveys of S.E. England and S. Yorkshire 1969-1985 (see Table 3.1) for rubella-specific antibodies. Details of the apparatus used and the reagents which were found to work satisfactorily are described in Appendices 1 & 2, respectively.

(a) Preparation and 'running' of gels

The basic radial haemolysis (RH) test procedure essentially follows the method reccommended by a Public Health Laboratory Service (PHLS) working party (Kurtz et al, 1980). The following describes the preparation and application of one 'test' and one 'control' gel:

All reagents are used, and each step of the procedure is conducted, at room temperature, unless otherwise stated. Agarose is dissolved in complement fixation buffer (CFB) in the proportion 1 g agarose to 100 ml CFB solvent, using an autoclave or a microwave oven. Two 15 ml aliquots of the 1% agarose sol are maintained at 55°C in a waterbath. Sheep red blood cells (SRBC), stored in alsevers for up to one week, are washed three times in CFB and accurately adjusted to a 15% suspension in CFB using a microhaematocrit or Wintrobe tubes. Into each of two 30 ml glass flat-bottomed universals, one marked test, the other control, is pipetted 0.3 ml of 15% SRBC. 0.3 ml of rubella haemagglutinin (at a titre of 128) is then mixed into the cells of the test universal only. The HA titre may be determined by titration (Pattison (ed.), 1982). For HA titres greater than or less than 128, the quantity used is adjusted proportionally.

After standing for 30 min (with periodic mixing) each bottle is filled with CFB, shaken, then centrifuged for 10 min at 2000 rpm. The supernatant fluids are discarded by inverting each universal and the cells are resuspended in 0.5 ml CFB (CFB plus Kanamycin at 100 μg/ml). At this stage the two 15 ml quantities of 1% agarose at 55°C are

transferred to a 43°C waterbath, i.e. approximately 5 min before being required (a precaution that prevents premature solidification of the agarose). 0.5 ml of undiluted Richardson's preserved guinea pig complement (freshly reconstituted) is mixed (gently) into the cell suspension in each universal. Two 10×10 cm petri dishes are labelled test and control and placed on a levelled surface near to the 43°C water bath. One after the other, the control and test universals are placed into the 43°C water for 10 sec, 15 ml of agarose is poured into each and gently swirled, and without delay, the contents are emptied into the appropriately labelled petri dish (ensuring that all sides are wetted). The gels are left to set for 10 min, after which they are stored in a humid box (inverted to prevent problems of condensation) at 4°C for 30 min or until used. Best results are obtained with gels less than a week old. If the RH plates are to be used within 2 days then the need for antibiotic in the CFB is obviated.

Serum samples to be tested are inactivated by heating for 20 min at 60°C in a water bath (first ensuring that the water temperature is evenly distributed and that sera are at the required temperature before timing begins). Using a 3 mm diameter steel borer connected to a suction device, 49 wells (7-8 μl capacity) are cut into each gel in a hexagonal configuration. Each inactivated serum sample is dispensed into the corresponding well (directed by a template) in the test and control gels using a haematocrit tube (replaced for each test serum). Nine of the wells are reserved; 8 (randomly chosen) for dilutions of a standard serum (see

below) and one for a rubella antibody negative control. Gels are placed in a humid box and incubated at 37°C for 24 h. The edges of the resultant zones of haemolysis are delineated immediately using a permanent marker (this is best achieved with side illumination against a black background or viewed towards a fluorescent light strip) and the diameters measured to the nearest 0.1 mm with a graduated eyepiece lens (×10 mag).

(b) Quantification of antibody concentration

A British Standard serum containing 360 international units (iu) of rubella antibody is obtainable from the National Institute of Biological Standards and Control (NIBSC), Holly Hill, London. A local standard serum was calibrated against this reference serum by the method of parallel line assay (Finney, 1978) and was estimated to contain 213 ± 19 iu/ml. For each gel in a test series (i.e. a batch of plates made and used together), serial twofold dilutions of the local standard, in rubella antibody negative serum, from undiluted to 1 in 128, were tested in 8 randomly located wells. An equation for the linear relationship between the logarithm of rubella antibody concentration and RH zone diameter (calculated using least squares regression analysis) was used to convert the haemolysis zone diameter of each test serum into standard rubella international units per ml (iu/ml). The sensitivity of the RH test was set at the highest standard dilution that consistently gave a visible zone of lysis.

(c) Within- and between-test variability

Random allocation of standards in each test plate avoided systematic variation, for instance, from uneven gel thickness (particularly that which results from agarose being drawn up towards the sides of the petri dish). The screening of a set of standard dilutions in each gel of a test series ensured that the standard 'curve' accounted for any between-gel variation.

Inter-test variability (that which arises between different batches of gels) was monitored by repeated screening (× 2 or 3) of 5 control serum samples, covering a wide range of rubella antibody concentrations, in each test series.

(d) Uncertain results

All RH negatives, and sera producing very feint test gel haemolysis zones, were screened again by RH for confirmation. Samples still giving equivocal zones of lysis were re-tested using an alternative serological technique (see below for details).

Some sera possess non-specific haemolysing activity that is not eliminated by heat inactivation. These sera contain anti-SRBC antibody and cause lysis both with antigen-coated (test gel) and uncoated (control gel) SRBC. A serum producing zones of haemolysis which are of equal diameter in both the test and control gels, and are of greater diameter than the test zone for the standard dilution with the lowest detectable concentration of antibody, may contain rubella-specific antibody whose activity is obscured by

non-specific haemolysis (NSH) resulting from SRBC antibody. Under such circumstances NSH was abolished by adding 50 μl packed SRBC to 200 μl of the test serum, incubating for 1h and re-testing by RH.

The hexagonal arrangement of wells cut into each RH gel (see Plate 1, rows C-E) gives a maximum well-to-well spacing of 15 mm. On the few occasions that this distance was inadequate and haemolysis zones overlapped preventing accurate measuremant, the sera were re-tested by RH.

(ii) Radial haemolysis test - Method II

A number of modifications to Method I were implemented prior to screening serum samples from the surveys of HLA tissue typed individuals (see Tables 5.2 & 5.3). These changes, listed below, are essentially ones of improved standardization of the technique necessitated by the methods used for assessing genetic associations with rubella antibody concentration (see Part 2 of Chapter 5) which require optimal reproducibilty of results. Apparatus and reagents required are found in Appendices 1 & 2.

(a) Basic procedure

Large re-usable 23×23 cm gel plates to hold 100 ml aliquots of agarose replace the small 10×10 cm petri dishes and 15 ml quantities of medium used in Method I. All other reagents are increased in the same ratio as for agarose, i.e. 100:15. A summary of the basic procedure for preparing and running RH gels by Method II is as follows:

2 ml of rubella HA (titre 128) is incubated with 2ml of 15% SRBC for 30 min in a 30 ml universal (periodically shaking). The antigen sensitized erythrocytes are then spun down (5 min at 2000 rpm) and the supernatant fluid is removed by aspirating. After washing in 25 ml CFB, spinning down for a further 5 min at 2000 rpm and removal of supernatant fluid, the coated SRBC are resuspended in 3.3 ml CFB (shaking thoroughly to separate agglutination 'clots'). 3.3 ml of complement is added to the cell suspension, the univerasl is immersed in a 43 °C water bath for 25 sec and the warmed mixture is then added to 100 ml agarose at 43°C in a 150 ml bottle. The contents are stirred briefly, but thoroughly, (making sure not to create bubbles) and immediately poured into a 23 x 23 cm plate labelled test. A control gel is prepared in the same way, with CFB substituting for HA. Gels may be stored at 4°C in a humid box but should be used within a working week.

When required, wells of 3 mm diamater are cut into the gels guided by a template of 144 hexagonally arranged dots. A diagonal well-to-well distance of 20 mm prevents any measuring problems from haemolysis zone overlap. Test sera are dispensed into test and control plates in 9 μl quantities using a micropipette with disposable tips. Gels are then incubated for 24 h at 37°C and read in the same way as before but taking care to ensure the coordinates of the haemolysis zone being measured are cross checked with the template to avoid confusion.

(b) Quantification of antibody concentration

A local standard, pooled from 10 high potency sera and calibrated against the 360 iu British Standard serum, was used to construct a standard 'curve', in each test series, to convert test serum zone diameters into antibody concentrations. Serial dilutions from undiluted to 1 in 256 were screened in each gel. The antibody concentration of the undiluted pooled standard was estimated, by parallel line assay, to contain 637 ± 38 iu/ml. This high potency exceded the concentration of more than 80% of all HLA typed specimens screened. The result was greater confidence in the rubella antibody concentrations determined from test zone diameters, because interpolation from the standard 'curve', rather than extrapolation, was the rule (Finney, 1978).

(c) Reproducibility measurements

In addition to monitoring between-test variation throughout the experimental procedure, using 5 control serum specimens of various rubella antibody levels, two one-off measuremants of the reproducibility of the test serum antibody concentrations were determined. Details of these tests are given in Chapter 5, Part 2 which examines the possibility of genetic association with rubella-specific antibody.

(iii) Other antibody test methods

Two other serological techniques, the haemagglutination inhibition (HI) test and the Rubazyme IgG ELISA (Abbott

Diagnostics, UK) were used in the experimental procedures. The HI method described in the PHLS monograph No. 16 (Pattison (ed.), 1982) was adopted using kaolin treatment (to remove non-specific inhibitors of haemagglutination) and day old chick red blood cells. The commercial ELISA was used according to the manufacturers' instructions.

Sera giving very feint test zones of haemolysis, not resolved by repeat RH screening, were confirmed by HI or ELISA. Furthermore, in the course of screening the samples from the survey of S.E. England (see Table 3.1) 33 and 42 RH negative samples (selected at random) were re-tested by HI and ELISA, respectively.

2.3 Characteristics of the Screening Methods

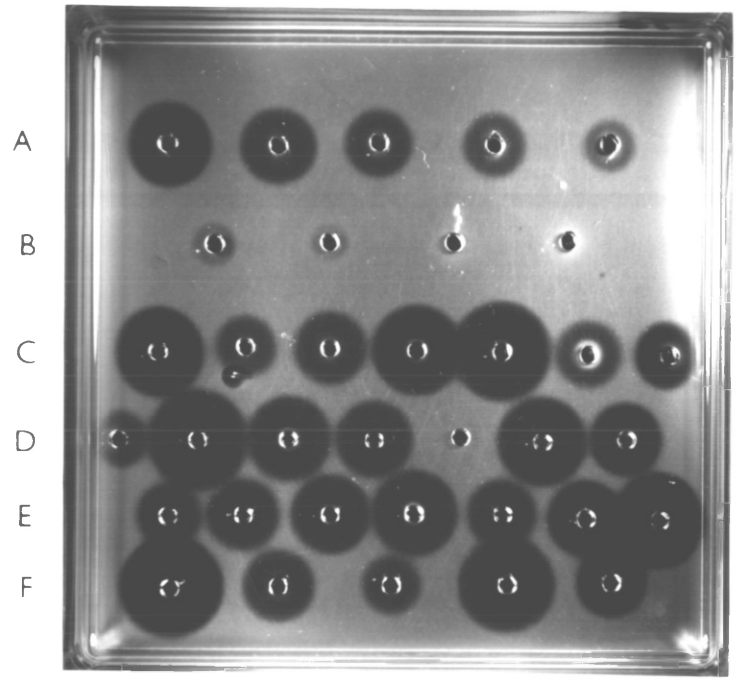
(i) Observations on gel quality and sensitivity to specific antibody

Although some week-to-week variation in the clarity of haemolysis in RH gels was a feature of the test, this did not prevent the measuring of zone diameters, and a level of sensitivity appropriate for a seroepidemiological screening tecnique could be maintained. A zone of lysis was routinely observable for the local standard serum diluted 1 in 64 (i.e. containing 3.3 iu/ml) in Method I, and for the pooled standard serum dilution of 1 in 256 (i.e. containing 2.5 iu/ml) in Method II.

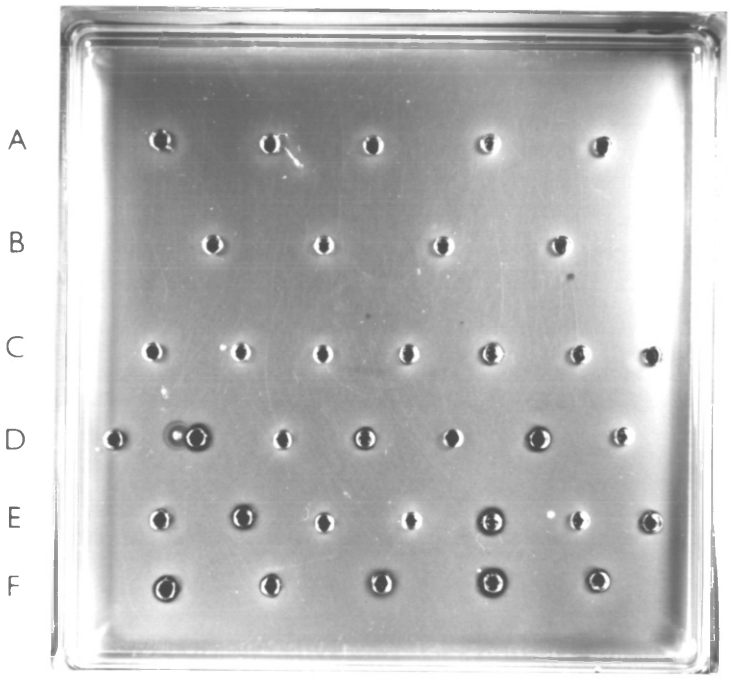
Plate 1 shows the quality of zones of lysis in a RH

Plate 1

RH Method I test and control gel (10x10 cm plates).



TEST



CONTROL

Rows A and B show successive twofold dilutions of a standard serum from undiluted (213 iu/ml) to 1/128 (1.7 iu/ml) and a negative control. Notice that the highest dilution of standard giving a readable zone of lysis is 1/64 (3.3 iu/ml). Rows C-E contain a random selection of test sera of unknown antibody concentrations. Row F contains 5 positive control specimens (from left to right they correspond to controls 1,3,5,2,4 recorded in Table 2.1).

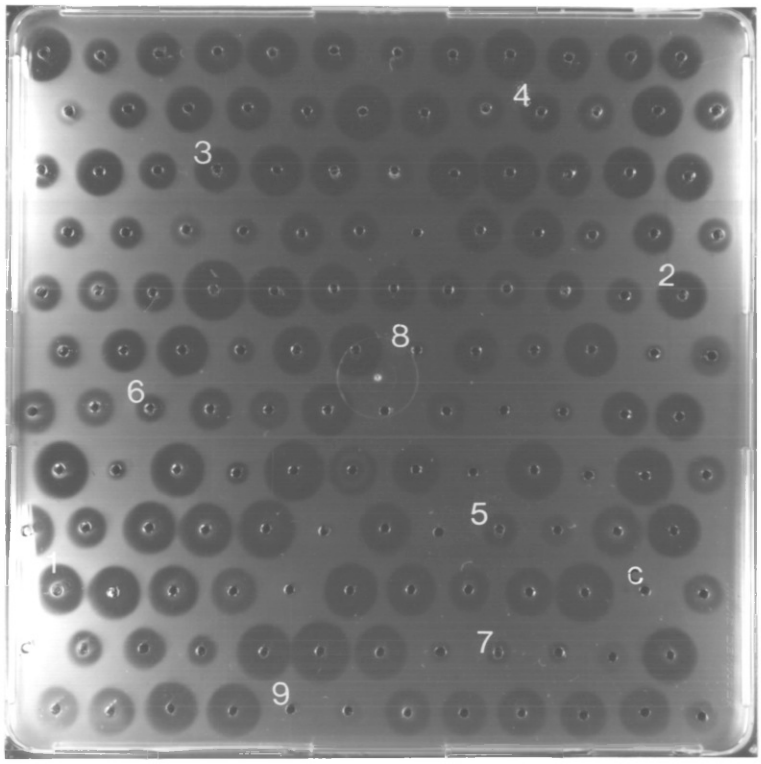
test gel produced by Method I, for a set of standard serum dilutions from neat to 1 in 128, a negative serum, 5 controls and 3 rows of randomly selected test sera. The control gel is shown alongside for comparison. A representative test and control gel produced by Method II is shown in Plate 2. Nine standards and a negative control serum are randomly distributed among 134 test sera.

Factors which undoubtedly contributed to limit variation in gel quality (and, thereby, increase standardization of the technique important to the reproducibility of results - see below) included (a) strict observance of the method procedure, (b) preparing and running gels, generally, in the same working week, and (c) using the same source of reagents, from the same batch (or lot) where possible.

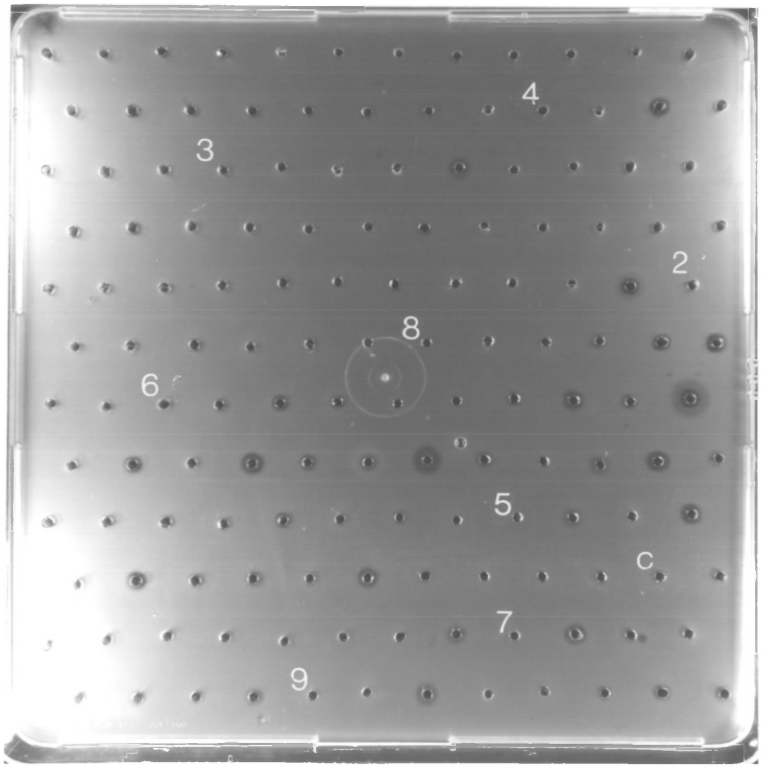
The degree of sensitivity, for both methods, is in accordance with previous evaluations of the RH technique (Kurtz et al, 1980; Neumann & Weber, 1983). A frequency distribution of antibody concentrations ($\log_e$ iu/ml) for 3310 serum samples from the survey of S.E. England, determined by Method I (with a sensitivity of 3.3 iu/ml) is shown in Fig. 5.1. Similar distributions (not illustrated) were obtained for all other surveys (Table 3.1, 5.2 & 5.3) using one or other of the methods. The lack of skewness and the rapidly diminishing tails at the extremes of the distribution suggest that few samples with low level antibody were mistaken as seronegative. Consequently, 3.3 iu/ml for Method I and 2.5 iu/ml for Method II were used as the cut off points for seropositivity for the analyses described in future chapters.

Plate 2

RH Method II test and control gel (23x23 cm plates).



TEST



CONTROL

Gels produced from a routine screen are presented. Doubling dilutions of a standard, numbered 1 (undiluted or 637 iu/ml) to 9 (a dilution of 1/256 or 1.25 iu/ml), and a negative control (c) are randomly dispersed amongst 134 test sera.

(ii) Antibody quantification: validity of the log-linear relationship

Representative plots of mean RH diameter (mm) against rubella antibody concentration (log iu/ml) for dilutions of the local standards used in Methods I and II, are shown in Fig. 2.1. Regression analysis yields the line of best-fit to each data set which may then be used to determine the antibody concentration of test sera from the diameter of zones of haemolysis (like those shown in Plates 1 & 2). The relationship between average RH diameter and the logarithm of serum dilution for 5 high potency samples (each point an average of 3 replicates) screened in a single test series (by Method I) is shown in Fig. 2.2. Linearity is maintained throughout the range of dilutions in each case (see also Clarke et al, 1977).

(iii) Reproducibility

Three sources (or levels) of variation have been considered; (i) within a gel, (ii) between gels of a test series, and (iii) between test series (i.e. between batches of gels run at different times). Variation occuring within a test series (sources (i) and (ii)) has previously been demonstrated to be small (Russel, McCahon & Beare, 1975; Neumann & Weber, 1983) and will be accounted for in the standard 'curve' for each particular test series.

Significant variation, however, is most likely to arise between test series (source (iii)). The use of standards

Fig. 2.1

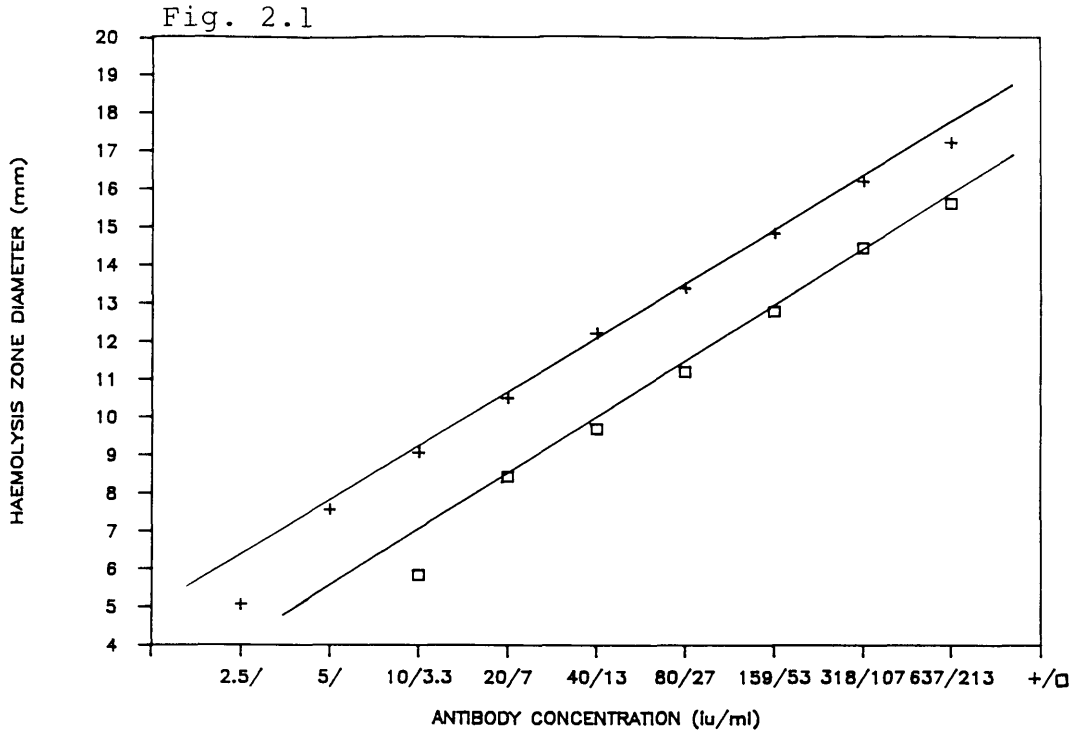


Fig. 2.2

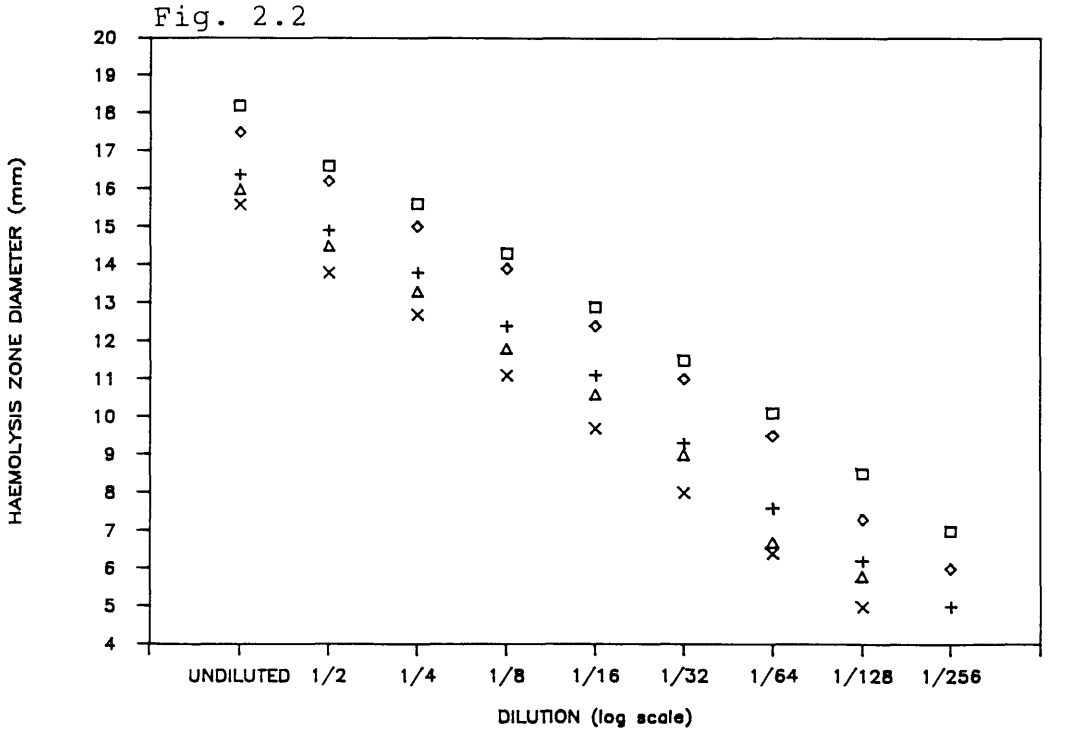


Fig.2.1 Relationship between haemolysis zone diameter and antibody concentration for serial doubling dilutions of two local standard sera used in Methods I (□) and II (+) of the radial haemolysis test (see text). The line of best-fit, calculated by regression analysis, is plotted through each set of observations (each point represents the average of five replicates).

Fig.2.2 Log-linear relationship between RH zone diameter and serum dilution for five high potency samples. Undiluted antibody concentrations for the serum samples are as follows: □, 1330; ◇, 1154; +, 624; △, 510; ×, 371. Each observation point is an average of three replicates.

reduces the effect of this variation on estimates of antibody concentration, because differences in zone diameter for test sera, which are of equal antibody potency, screened on separate occasions, should be reflected in zone diameters for the standards.

The results of monitoring inter-test variation for Method I and Method II are recorded in Table 2.1a & b, respectively. For both methods the variance (s^2) and the 95% confidence limits (CL) of the mean remain relatively constant and independent of antibody concentration. Inevitably, therefore, the coefficient of variation (CV%) shows a tendency to increase with decreasing antibody concentration, but the average CV% (weighted by the number of test series) remains less than 10. An improvement in the reproducibility of the technique following modifications that comprise Method II is suggested by the lower average CV% for Method II compared with Method I, and the greater confidence (in general) in the estimated mean antibody concentration for the controls in Method II.

(iv) The effect of freeze-thaw action and storage duration on serum antibody potency

Characteristics of the samples and sources used in this seroepidemiological project are detailed in Tables 3.1, 5.2 & 5.3. Specimens from the virology departments had inevitably been frozen and thawed on a number of occasions, and this was known to be the case, in particular, for serum samples from Kings College Hospital (part of the survey of

Table 2.1

Inter-test variability for the radial haemolysis test for 5 control sera with various antibody concentrations.

(a) Method I

	Control				
Statistic	1 High	2	3	4	5 Low
Mean	7.08	6.40	4.67	4.05	3.17
S^2	0.231	0.223	0.248	0.128	0.184
CL	0.40	0.39	0.42	0.30	0.36
# test series	8	8	8	8	8
# replicates	22	22	24	24	24
CV%	6.79	7.38	10.68	8.85	13.29

Average CV% (weighted by # test series): 9.40

(b) Method II

	Control				
Statistic	1 High	2	3	4	5 Low
Mean	7.19	6.37	4.57	4.12	3.06
S^2	0.197	0.133	0.144	0.105	0.131
CL	0.41	0.26	0.27	0.30	0.26
# test series	7	10	10	7	10
# replicates	15	22	23	15	23
CV%	6.18	5.73	8.30	7.85	11.80

Average CV% (weighted by # test series): 8.10

Mean = Mean antibody concentration ($\log_e$iu/ml)
S^2 = variance
CL = 95% confidence limit
CV% = coefficient of variation

S.E. England, Table 3.1). The period of cold storage of samples, prior to screening for rubella antibodies, varied from less than 6 months (e.g. HLA samples from the London Hospital, Whitechapel - Table 5.3) to over 15 years (S. Yorkshire, 1969 - Table 3.1).

To obtain an estimate of the robustness of rubella-specific antibody, detectable by RH, to these two factors, 5 serum samples of various antibody concentrations were thawed, held at room temperature for about 2 h, and then re-frozen at $-20\ ^{o}C$, on 10 separate occasions over a period of 1 year. The samples were screened in triplicate at the onset of the experiment and again after the time period had elapsed. To distinguish any loss of activity resulting from storage rather than thawing, an aliquot of each sample was kept at $-20^{o}C$ for the duration of the experiment (without thawing) and screened in parallel to the frozen/thawed samples.

The results are shown in Table 2.2. No significant change in antibody concentration, as a result of storage or of thawing, is observable. However, the conditions of the experiment might, perhaps, be criticised for not being sufficiently extreme in both the number of freeze/thaws and, in particular, the storage duration (the experiment is continuing).

(v) RH false negatives and equivocal results

No discrepant results were uncovered by retesting 76 RH negative sera (comprising approximately 20% of all negatives

Table 2.2

Effect of freeze-thaw action and prolonged cold storage on antibody concentration in 5 control sera.

Control	Mean Antibody Concentration (3 replicates) + 95% Confidence Limits		
	Before 15/11/84	After 14/11/85	
		Stored at -20 C Thawed 10 times	Stored at -20 C
1 High	7.11±0.81	7.22±1.48	7.11±0.55
2	6.18±0.84	6.42±0.86	6.54±0.77
3	4.43±0.57	4.51±1.15	4.65±0.43
4	3.88±0.83	3.97±1.01	4.06±1.02
5 Low	3.19±0.85	2.98±0.66	3.07±0.42

for the S.E. England survey) by HI or ELISA. This confirms the general observation that the frequency of false negatives is very low (Morgan-Capner, 1984). Nevertheless, the occurrence of sera which give a false negative result due to a RH 'blocking factor' has previously been noted (Champsaur, 1983; Kangro, Campbell-Benzie & Heath, 1983). Two samples from the survey of S.E. England, though RH negative, were observed to inhibit haemolysis of adjacent positive sera in test gels. Both sera proved to contain rubella specific IgG (>15 iu/ml) by Rubazyme ELISA. The origin of a 'blocking factor' is considered further in Chapter 5, Part 2 in relation to inherited characteristics.

Table 2.3 records the number of specimens, by sample source, which gave very feint RH test zones and were subsequently resolved by HI or ELISA testing (except samples from the survey of S. Yorkshire 1973 for which the condition of the specimens was too poor to warrant re-treatment, and for HLA typed samples - see section 5.7). Duration of cold storage was observed to have an adverse effect on the quality of haemolysis, which is reflected in the number of equivocal results from surveys such as S. Yorkshire 1976, 1973 and 1969.

2.4 Discussion

Perhaps the single most important characteristic commending the radial haemolysis technique for use in seroepidemiological surveillance is its ease of operation.

Table 2.3

Frequency of equivocal RH results following screening of samples from all sources using Method I or II. (Other details on samples and sources in Tables 3.1 & 5.2).

Sample source	RH Method	Year bled	Number equivocal	% equivocal (sample size)
Kings College Hospital	I	1980	21	1.34 (1562)
S. London BTC	I	1984	9	0.49 (1823)
Sheffield PHLS	I	1969	9	1.33 (675)
		1973	45	8.05 (559)
		1976	5	1.44 (347)
		1979	0	0.0 (407)
		1981	2	0.51 (394)
		1985	1	0.23 (443)
SWBTC	II	1984-6	1	0.27 (368)
SEBTC	II	1984-6	0	0.0 (477)
CRC	II	1981-4	0	0.0 (152)
JRH	II	1977	0	0.0 (177)
LHW	II	1984-6	2	0.35 (575)

The methods (I & II) are simple to follow, requiring the use of unsophisticated apparatus generally available in research and diagnostic laboratories. Test sera (used in microlitre quantities) needed only one pre-treatment, that of heat inactivation (compare with the HI test). Consequently, working alone and under ideal conditions, it was possible to screen (i.e. pre-treat sera, and prepare and run gels, but not confirm equivocal results) between 400 and 500 serum samples in a week.

As with any serological test some variability was observed in the RH results. Considerable importance was placed on the standardization of Method I and II in order to limit this variation (within- and between-test series) arising from the experimental procedures. The outcome was a high degree of reproducibility of the quantitative results (see Table 2.1 and the results in Chapter 5, section 5.6(ii)) and a sensitivity to low level antibody which could be sustained. Good reproducibility of results adds authenticity to the analytical conclusions made in later chapters, and antibody measurements in standard units (iu/ml) enables the comparison of results from different surveys.

Some reduction in inter-test variation was obtained for Method II when compared with Method I, but this was accompanied by an increased cost of the screening process (approximately twofold). For most serological survey work the increased reproducibility of Method II would not justify the elevated cost. However, the analyses of genetic association with antibody concentration, carried out in

Chapter 5, Part 2, did warrant its use.

A high degree of sensitivity to rubella-specific antibody was routinely obtained using either of the RH methods developed (3.3 iu/ml for Method I and 2.5 iu/ml for Method II). It is possible to enhance the technique in order to detect antibody levels as low as 1-2 iu/ml, by, for example, double filling wells (increasing the quantity of serum used), lengthening the gel incubation period, or decreasing the concentration of SRBC or HA (Champsaur & Slim, 1981; Neumann & Weber, 1983; Morgan-Capner, 1984). However, the log-normal distribution of antibody concentrations for a population of individuals (see Fig. 5.1) suggests that an insignificant number of low seropositives would be uncovered by sensitivity improvements of this nature, and, furthermore, such modifications would undoubtedly incur either a time forfeit on the screening process or cause a reduction in the haemolysis zone clarity. Consequently it was deemed unnecessary to incorporate changes in the methods to improve on the test sensitivity.

Some uncertainty exists in the literature as to the interpretation of low positive antibody concentrations in relation to rubella immunity (Pattison, 1982; Banatvala, 1985). For the purpose of deciding whether or not to administer rubella vaccine, individuals with detectable antibody which is less than 15 iu/ml are regarded as non-immune (Mortimer et al, 1981). The cutoff point of 15 iu/ml for immunity designation was originally selected to compensate for a deficiency in the HI test (Bradstreet et al, 1978), and has remained with us in spite of more

reliable and sensitive techniques like RH (there is of course some justification in erring on the safe side when the consequence of a mistake might be a congenitally deformed child).

However, for the seroepidemiological survey employing a screening technique able to reliably detect antibody concentrations much lower than 15 iu/ml, there is no justification for such conservatism. Any detectable level of naturally induced anti-viral antibody indicates the presence of memory cells which enable the individual to mount a secondary immune response on re-exposure to the virus (Wagenvoort et al, 1980). Viraemia and the subsequent state of infectiousness should, therefore, be averted, thus preventing vertical or horizontal transmission.

No conclusive evidence exists to contradict this supposition. Studies of natural or experimental rubella virus challenge in individuals with low level naturally-induced antibody have not reported the detection of significant levels of IgM (the antibody class associated with primary infection) or of virus excreted into nasopharyngeal secretions (Mortimer et al, 1981; O'Shea et al, 1983; Morgan-Capner et al, 1985). Evidence also indicates that low level pre-existing antibody in pregnant women, acquired by natural rubella infection, is likely to prevent intra-uterine infection and foetal damage on re-exposure to rubella (Craddock-Watson et al, 1981; Morgan-Capner et al, 1985).

It seemed quite appropriate, therefore, that in the analysis of results in this study, the threshold of

sensitivity of the RH technique should be inferred as the threshold of rubella immunity.

The value of low level vaccine-induced antibody, on the other hand, in relation to immunity is questionable. This issue is considered in Part 1 of Chapter 5 in the context of average antibody concentrations in a vaccinated population.

Proportionally very few of the 7000+ samples screened by the RH methods in this study produced equivocal results and the majority of those that did were presumed to have resulted from poor storage conditions. Furthermore, as long as heat inactivation of sera was conscientiously carried out, the incidence of non-specific haemolysis was insignificant. As with previous evaluations, the RH test proved to be reliable in relation to the differentiation of seropositivity and seronegativity. Therefore, although some discrepant results did arise, the requirement for backup tests (HI and ELISA) is questionable. Instead there seems to be a case, in future surveys using the RH test, for simply eliminating the few equivocal results from further analysis. This would be time saving and (as long as large numbers of samples were involved) unlikely to incur significant bias.

In summary the RH technique was found to be simple and rapid to perform, inexpensive to setup and run, and could be effectively standardized to provide reproducible quantitative results. The technique was reliable in the discrimination of positive from negative sera, and sensitive to low level rubella-specific antibody. The threshold of sensitivity for Method I (3.3 iu/ml) and Method II (2.5

iu/ml) have been regarded as the cutoff points for immunity in the analysis of results in the following chapters. The RH test is, today, a thoroughly evaluated test and its growing acceptance as the method of choice for serological surveys seems to be justified.

Chapter 3

RUBELLA IMMUNITY AND INFECTION RATES IN ENGLAND: SEROLOGICAL EVIDENCE

Part 1

OBSERVATIONS FROM AGE-SEROLOGICAL PROFILES

3.1 Introduction

The first part of this chapter deals with empirical (i.e. non-mathematical) analysis of seroepidemiological data for rubella acquired from a number of horizontal cross-sectional serological surveys in England (Table 3.1). The intention is to obtain information on the state of rubella immunity across a wide band of age classes (from new-born children to the elderly) in the population, and to determine what impact the current immunization programme has had on levels of immunity (particularly in females but also in the male community).

Serum samples from the surveys documented in Table 3.1 were screened for the presence of rubella-specific antibodies using the radial haemolysis test - Method I (see Chapter 2). The empirical data thus provided form the backbone of the seroepidemiological analyses presented in

this and forthcoming chapters.

Conclusions drawn from the following observations in the first part of this chapter and analyses in later sections should be made in light of the nature of the raw data utilised (i.e. the characteristics of the surveys upon which they are based). Some information is therefore provided in the next section on the method of collection of serum samples, on the sample populations from which they were drawn, and on possible limitations of their use in epidemiological research.

3.2 Collections of Serum Samples: Characteristics and Limitations

A central component of many seroepidemiological investigations of infectious disease transmission is a horizontal cross-sectioned serological survey. Ideally this should be based upon a collection of serum samples obtained by random selection from a defined population at one point in time. Specimens should be taken from individuals over a wide range of age classes (finely stratified, with equal numbers in each age group) for whom information on sex, socioeconomic status and origin is available.

In reality, practical considerations such as the availability of samples are likely to be of equal importance in determining the exact nature of serum collections utilised in epidemiological study. This is certainly true for the sets of samples used in this project,

Table 3.1

Survey description: South East England and South Yorkshire

Survey or catchment area	Source of samples	Reason specimen taken	Year bled	Number of Sera	Details: age (yrs)	sex
S.E. England ie S. London & the Home Counties	i Kings [1.] College Hospital, London	Virological & antenatal rubella screening	1980	1562	0-92	yes
	ii S. London [1.] Blood Transfusion Centre	Blood doning	1984	1823	18-65	yes
S. Yorkshire ie Barnsley Sheffield, Rotherham, Doncaster	Sheffield [3.] Public Health Laboratory	Virological & antenatal syphilis screening	1985	443	1-93	yes
			1981	394	1-96	no
			1979	407	1-91	no
			1976	347	1-93	no
			1973	559	1-89	no
			1969	675	1-89	yes

Collaboration in the collection of samples was obtained from:

1. Dr. P. Morgan-Capner;
2. Ms. B. Cant;
3. Dr. R. Jennings

the characteristics of which are detailed in Table 3.1. Selection of specimens was made, as far as possible, according to the guidelines outlined above. However, finite resources (of time and money) were the overriding factors which dictated the sources utilised. These sources included hospital virology departments and a blood doning centre, for which sera were readily available in large numbers.

The catchment areas for the various sources give rise to two (geographically) distinct study areas or populations, namely South East England (which includes South London and the home counties of Kent, Surrey and East and West Sussex) and South Yorkshire (by which is meant the conurbation of Sheffield, Barnsley, Rotherham and Doncaster). Specimens had already been collected, and stored for various lengths of time, for the purposes of either microbiological investigation (Kings College Hospital and Sheffield Public Health Laboratory) or for blood group testing (South London Blood Transfusion Centre).

Samples from the two S.E. England sources (Table 3.1) were collected as complementary sets, and for the purposes of analyses have been pooled. There is justification for this in terms of their similar catchment areas and the relatively short time interval between their original collection (less than the inter-epidemic period for rubella). The yearly surveys of S. Yorkshire were kept as such in order to investigate longitudinal trends.

It is important to mention the limitations of these sample collections as sources of epidemiological data:

(i) it cannot be stated, with any degree of certainty, that the surveys detailed in Table 3.1 represent random samples from the two study populations (nor of the country as a whole);

(ii) furthermore, they do not necessarily represent an unbiased cross-section of all social or economic classes, and include samples (numbers unknown) from people whose origin or birthplace is outside the UK;

(iii) the study areas include urban and rural parts;

(iv) finally, not all age classes or sexes are equally represented. Difficulty was experienced in obtaining specimens from children, particularly those less than 10 years of age, and, in general, samples from females were more easily acquired (blood donors and, of course, antenatal patients).

These points raise doubts about how representative results from the analyses are for their respective study areas or of England in general. Specimens have not been segregated according to the factors given in points (ii) and (iii). This is partly because of an incomplete record of such information, and the subjectivity of this process (for instance, people may live in rural areas and commute to work in an urban location, or vice versa), but also because subsequent analyses would be based on much reduced sample sizes, thereby diminishing the chance of obtaining statistically significant results.

There is no denying that factors such as city or country life, and perhaps social class and ethnicity will affect the transmission dynamics of an infectious disease agent (e.g.

rate of acquisition of infection) (see Anderson, 1982).

Nevertheless, there was adequate justification in using these samples (because of the relative ease with which they could be obtained) as a first attempt to achieve the aims of the study. The collections of samples shown in Table 3.1 do approximate to the requirements of cross-sectional horizontal surveys; samples were collected over short intervals of time, for a wide age range, and in most cases information on sex was available. It is also unlikely that the surveys dramatically deviate from random collections from the study populations; most of us give samples of blood for virological investigation at some point in our lives, and, contrary to popular belief, people who donate blood (at least in the South East) come from all walks of life, e.g. factory workers, office staff, industry, and the professions (B. Cant, S. London BTC, personal communication). Most importantly, these surveys should reasonably serve as the basis of a 'pilot study' and achieve the broad aims of the project. This should, hopefully, stimulate the interest in and recognition of the importance of serological surveys in epidemiological research.

3.3 Immun ity to Rubella in Two Study Populations

(i) South East England

The change, with age, in the proportion of males and females serologically positive for rubella-specific antibody (i.e.

$\geq$ 3.3 iu/ml, by radial haemolysis - Method I) in the combined samples obtained from two S.E. England sources (see Table 3.1) is recorded in Fig. 3.1.

The proportion of the population who have experienced infection rapidly rises over the childhood and teenage years, such that over 85% of individuals possess rubella antibodies at the age of 20 years. Thereafter the profiles level out. Approximately 95% immunity is attained in those greater than 40 years and 97.5% are seropositive above the age of 60 years.

Error bars, indicating 95% confidence limits (CL) for the proportion serologically positive in each 5 year age class, are determined under the assumption that seropositivity is binomially distributed. With mean, p, (i.e. the proportion immune), and variance, p(1-p),

$$CL = p + CI(95\%)$$

$$CI(95\%) = t.SE$$

$$SE = (p(1-p)/n)$$

where, CI(95%) is the 95% confidence ineterval

t is the Student t statistic at $\alpha = 0.05$ (1 tail)

n is the sample size.

It can be seen from Fig. 3.1 that the smallest confidence limits occur when sample sizes are largest (i.e. upper age classes), but their magnitudes are large where changes in the proportion immune, with age, are most rapid (i.e. < 20 years).

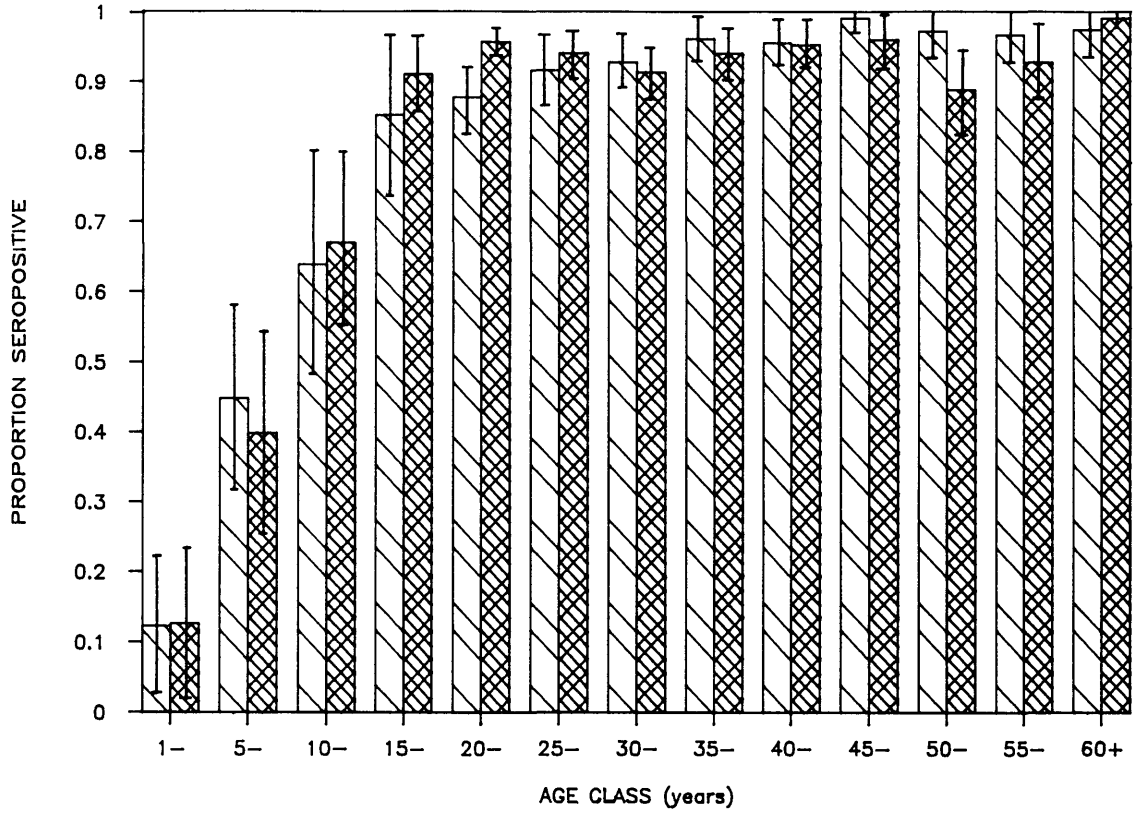


Fig.3.1 Age-serological profile for rubella in South East England (1980/84). The graph records the changes, with age, in the proportion of males (▨) and females (▩) seropositive for rubella from a survey of S.E. England between 1980 and 1984. Error bars indicate 95% confidence limits (based on the positive binomial distribution). Sample sizes in each age class, for males and females respectively, are as follows: 1-4, 48/39; 5-9, 60/50; 10-14, 39/61; 15-19, 48/137; 20-24, 141/310; 25-29, 146/259; 30-34, 169/235; 35-39, 163/207; 40-44, 138/151; 45-49, 126/103; 50-54, 110/100; 55-59, 93/84; 60+, 124/127.

The male and female profiles are broadly similar. However, the acquisition of anti-rubella antibodies by males is consistently slower than for females over the range 10-14 to 25-29 years of age. Over 95% of females are immune by 20-24 years of age; such a level of immunity is not attained by males before the 35-39 year age class. A significant difference, at the 5% level, between male and female immunity is indicated by non-overlapping error bars for the age class 20-24 years.

Raised levels of seropositivity in females compared with males, between the ages of 10 and 29 years, reflect the impact of vaccination of schoolgirls aged 11-13 (and after 1978, 10-15) years, since 1970. The selective programme of immunization has built on the level of natural immunity acquired from infection early in life, causing a significant reduction in the proportion of seronegative females entering the years of childbearing (roughly 15-40).

This is shown more clearly in Fig. 3.2, which records the proportions of seronegative men and women for the age classes 15-19 to 35-39 years. The effect of vaccination is most obvious in the ages between the years 15-19 to 25-29. For instance, there is a 65% reduction in susceptible females aged 20-24 years, compared with that which prevailed before vaccination. Here it is assumed that herd immunity for males, shown in Figs. 3.1 & 3.2, is representative of the level of immunity for females pre-1970, (indeed, limited serological data for the prevalence of rubella antibodies in females for London, England, prior to immunization, endorses this assumption (Rawls et al,

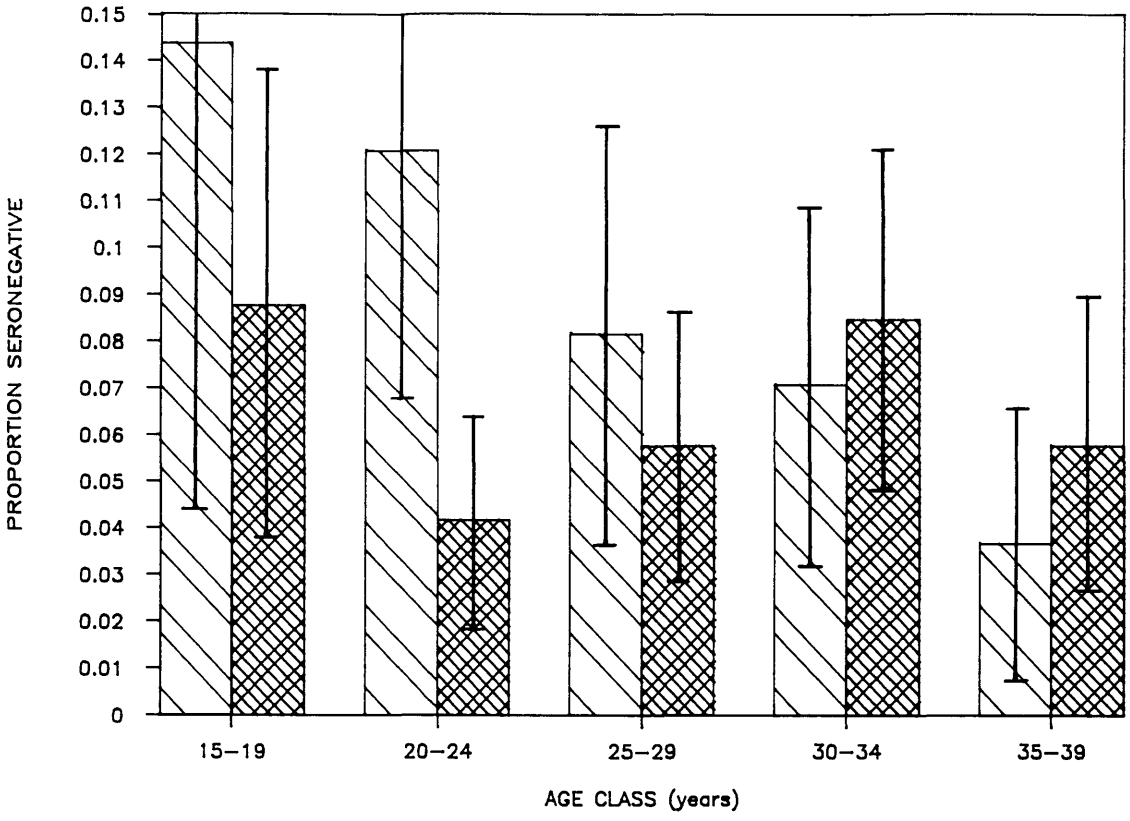


Fig.3.2 Proportions of males (▨) and females (▩) serologically negative for rubella in S.E. England 1980/84. Only results for the critical childbearing years are illustrated. Sample sizes and 95% confidence limits are as defined in Fig. 3.1.

1967)).

It can be seen from Fig. 3.2 that there is an increase in the proportion of seronegative women in their late twenties and early thirties. This is interpreted as a return to pre-vaccine levels of immunity, as female ages exceed those of the first vaccinated schoolgirl cohorts.

Throughout the age range shown in Fig. 3.2 the proportion of susceptible males declines. There is a 10% reduction in seronegativity between the age classes 15-19 (approximately 15% non-immune) and 35-39 years (less than 5% non-immune). This suggests that rubella is not uncommon in males of this age, representing a potential risk to non-immune pregnant mothers.

The decline in maternally derived antibody in neonates is illustrated in Fig. 3.3. Sample sizes are not large (note the height of the error bars); serum samples for infants are not easy to obtain. The figure shows that the average persistence of detectable meternal antibody is between 3 and 6 months, and has become undetectable in all subjects by the end of the first year of life. Similar rates of decay were recorded for a survey in Manchester, England in 1976-77 by Craddock-Watson (see Anderson and May, 1983).

(ii) South Yorkshire

The age-serological profiles for rubella derived from samples obtained from South Yorkshire (Sheffield, Barnsley, Rotherham and Doncaster conurbation, see Table 3.1), are

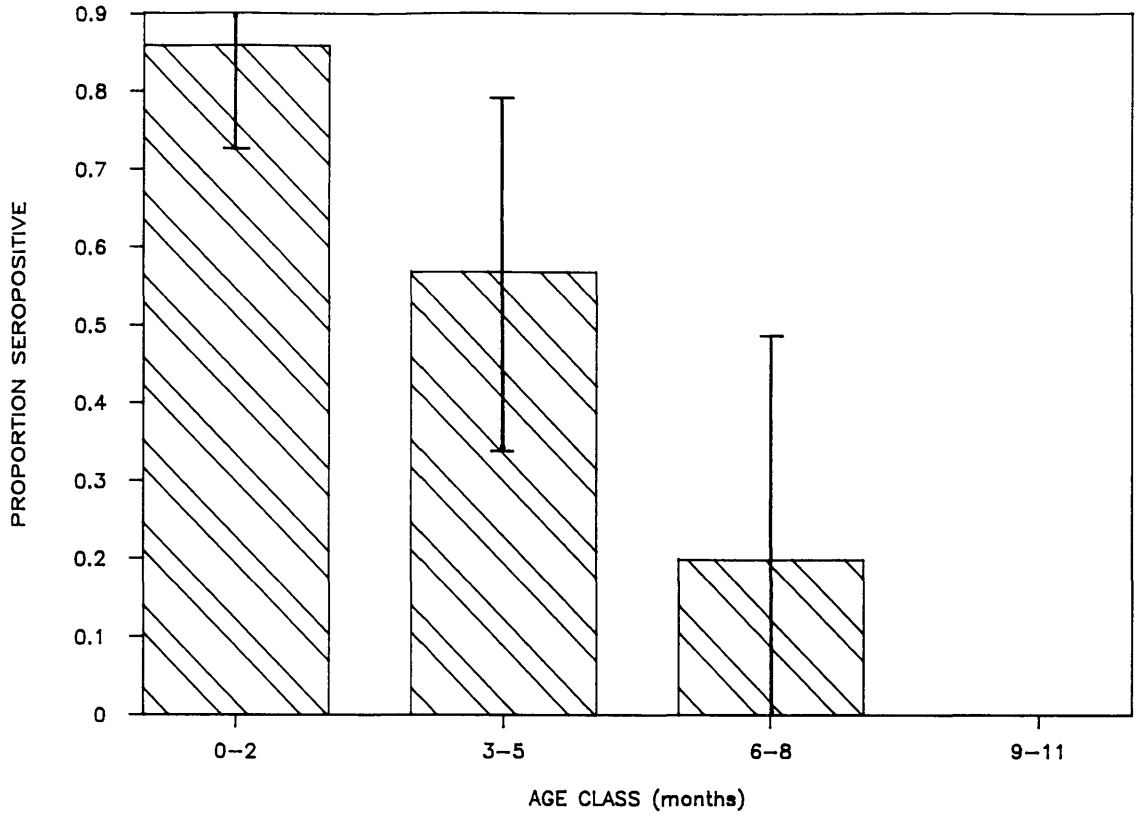


Fig.3.3 The decay in maternally derived antibody for neonates (males and females totalled) in their first year of life from S.E. England 1980. 95% confidence limits are illustrated. Sample sizes for each three month age class are: 0-2, 28; 3-5, 21; 6-8, 10; 9-11, 7.

presented for the years 1969, 1976, 1979, 1981 and 1985, in Fig. 3.4. No segregation for sex is shown and each data point is derived from a sample size of 25 (unless otherwise indicated). The results for 1973 are not recorded because of deterioration of antibody in samples due to poor storage (see Chapter 2).

The profile for the pre-vaccination year, 1969, and those for sequential years post initiation of immunization, (1976-85), are all remarkably similar. Approximately 90% of all individuals are serologically positive for rubella by age 20 years. This observation supports the frequently stated remark that the current rubella immunization policy will have little impact on the overall transmission of rubella virus in the community (Hinman et al, 1983; Noah, 1983; Anderson & May, 1983).

The bar graph of Fig. 3.5 compares the results (total of males and females) for 1969 with those of 1985. Even after 15 years of selective immunization there is no consistent difference observable between the two profiles.

Data for 1969 and 1985 have been analysed by sex and the resultant age-serological profiles are illustrated in Fig. 3.6a & b, respectively. Sample sizes are, in general, very small (see legend to figure). Nevertheless, some interesting and significant trends are to be observed.

In the pre-vaccination (1969) results, the age at which males acquire immunity seems consistently younger than females over the age range 1-29 years (Fig. 3.6a), although the differences in seropositivity levels are not statistically significant (at the 5% level).

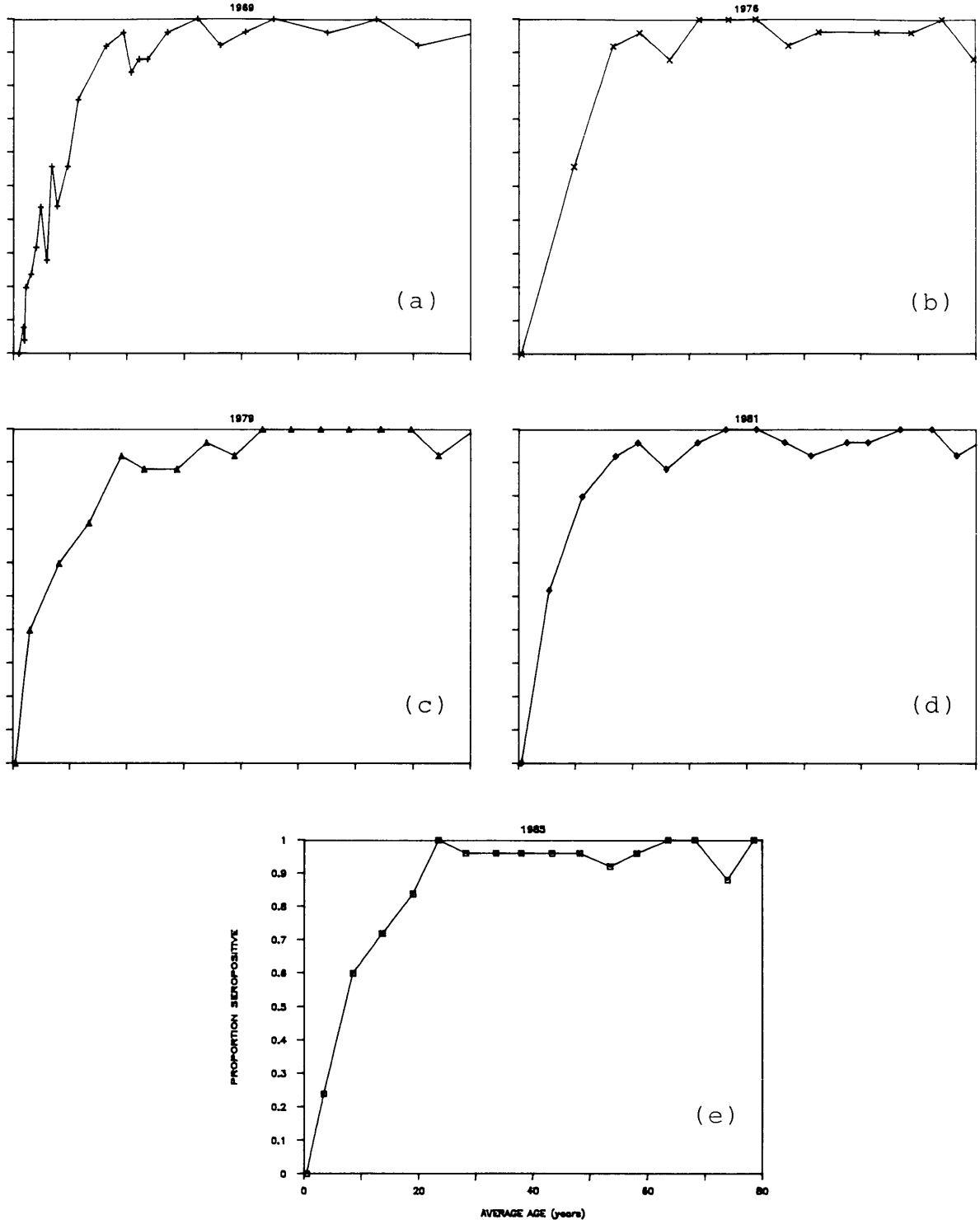


Fig.3.4 Rubella immunity in South Yorkshire for 1969 and for a number of years after the onset of selective immunization. Changes, with age, in the proportion serologically positive for rubella in S. Yorkshire are shown for surveys from 1969, 1976, 1979, 1981, 1985 (graphs a - e, respectively). Each data point represents a sample size of 25 individuals (males and females totalled).

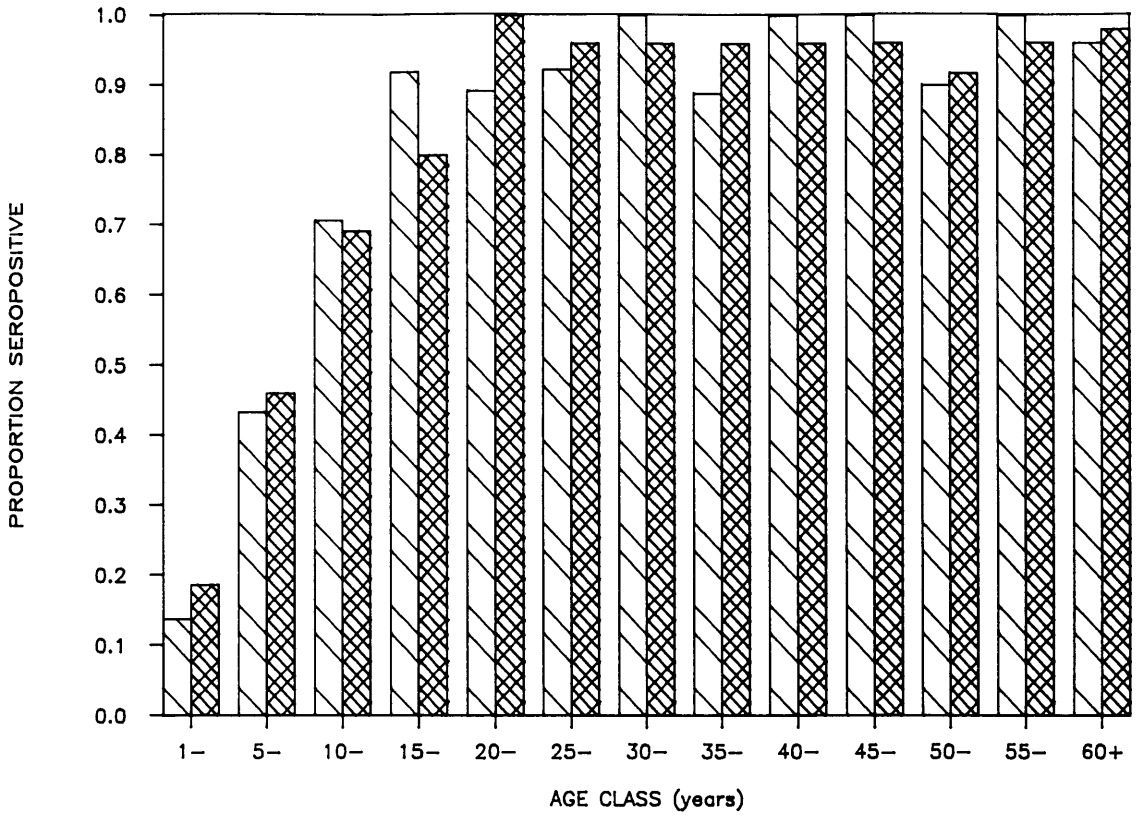


Fig.3.5 Comparison of rubella age-serological profilesfor 1969 (▨) and 1985 (▩) from S. Yorkshire. Sample sizes, for 1969 and 1985 respectively, are as folows: 1-4, 180/16; 5-9, 106/26; 10-14, 41/26; 15-19, 37/25; 20-24, 92/25; 25-29, 13/25; 30-34, 34/25; 35-39, 27/25; 40-44, 30/25; 45-49, 16/25; 50-54, 10/24; 55-59, 12/26; 60+, 76/150. No significant difference was observed between the proportion seropositive for 1969 and that for 1985 within any age class.

Post-vaccination (1985) proportions of males and females serologically positive for rubella in the age classes 1-4 and 5-9 years (Fig. 3.6b), mirror those for pre-vaccination (1969) children (Fig. 3.6a). However, in contrast to pre-vaccination results, the prevalence of female seropositivity, between the ages of 10 and 19 years, in 1985 exceeds that for males of the same age. Indeed, the difference is significant at the 5% level for the 10-14 year age class.

In general, as a result of vaccination since 1970, female herd immunity in South Yorkshire 1985 (Fig. 3.6b) has increased over that which prevailed in 1969 (Fig. 3.6a), for the age range 10-29 years. Comparison of the proportion of females seropositive in 1985 with that for 1969 in the 10-14 year class gives an estimated reduction of 77% in the proportion of seronegative females entering the 'at-risk' childbearing years.

Conversely, for ages 10-19 years the proportion of males immune to rubella in 1985 has declined from its pre-vaccine level.

Caution must be applied when interpreting these data because the sample sizes involved are small. However, there is evidence of a tendency for males to acquire rubella antibodies earlier in life than females, and, tentatively, it may be inferred that the reduction in the number of infectious females in the community as a result of selective immunization, has caused a concomitant reduction in the rate at which males, of the same age, acquire infection.

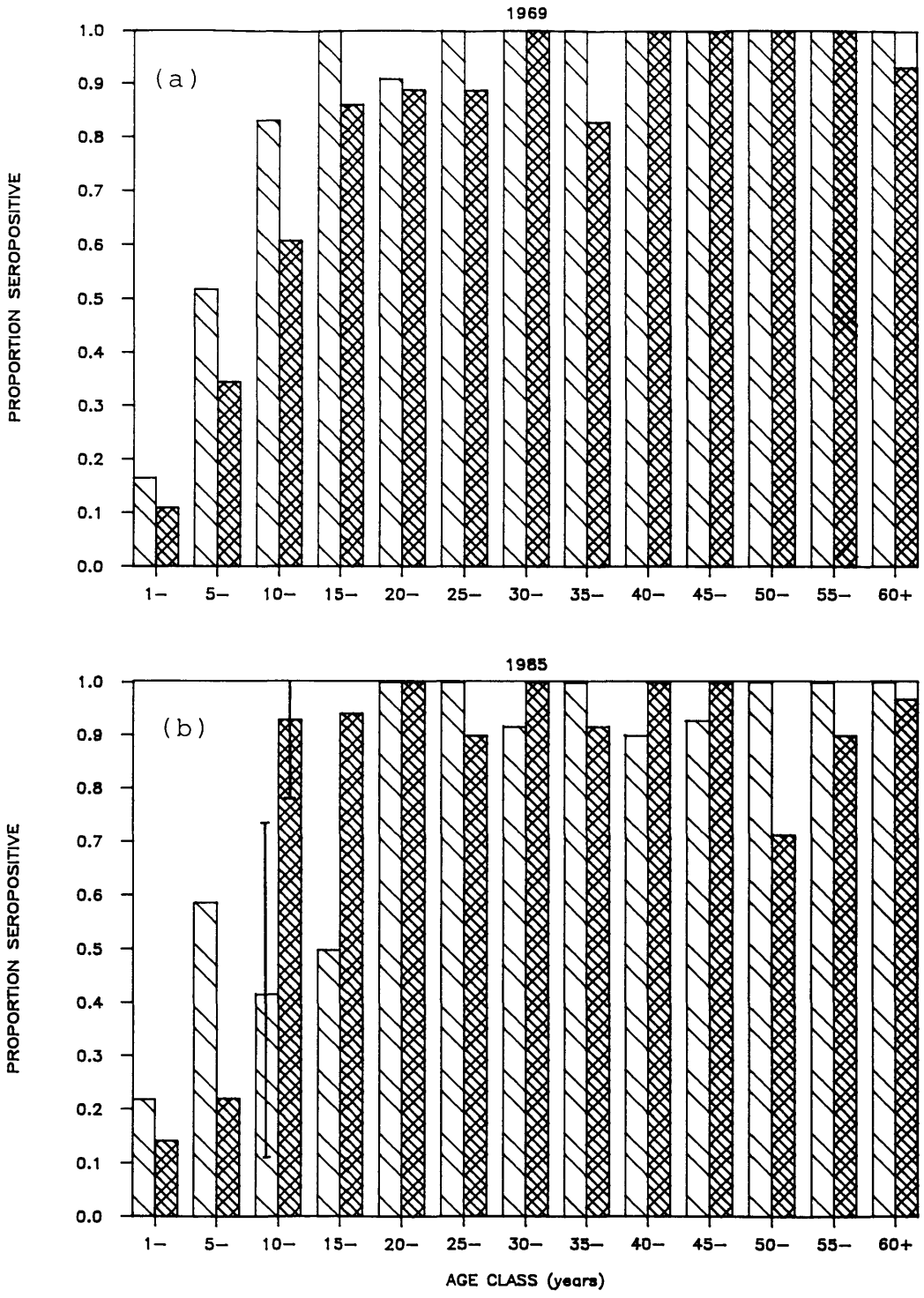


Fig.3.6 Age- and sex-stratified serological profiles for rubella from S. Yorkshire for 1969 (graph a) and 1985 (graph b). Sample sizes in each age class for males (▨) and for females (▩) respectively are:
1969: 1-4, 96/82; 5-9, 54/52; 10-14, 18/23; 15-19, 8/28; 20-24, 11/81; 25-29, 3/9; 30-34, 9/24; 35-39, 9/18; 40-44, 7/10; 45-49, 6/5; 50-54, 3/2; 55-59, 4/5; 60+, 22/15.
1985: 1-4, 9/7; 5-9, 17/9; 10-14, 12/14; 15-19, 8/17; 20-24, 7/18; 25-29, 14/10; 30-34, 12/13; 35-39, 13/12; 40-44, 10/13; 45-49, 14/9; 50-54, 17/7; 55-59, 16/10; 60+, 55/94.
95% confidence limits are shown for age classes only where overlap of error bars between sexes did not exist.

(iii) Comparison of S.E. England and S. Yorkshire

The age-serological profile (ASP) for S.E. England 1980/84, is compared with the profile for S. Yorkshire, 1969, in Fig. 3.7a, and 1985 in Fig. 3.7b, (male and female samples are pooled in each case). The value of relatively large sample sizes, as for the S.E. England survey when compared with S. Yorkshire, is illustrated by the size of the error bars for each survey. This is reflected in a greater degree of confidence in the results and conclusions derived.

An overall similarity in the change, with age, in the proportion seropositive, from both study areas, is evident from the histograms. Approximately 90% of individuals are serologically positive for rubella specific antibodies by the 20-24 year age class.

However, the average age at acqisition of infection is earlier in South Yorkshire (1969 and 1985) than in S.E. England 1980/84. This is illustrated by the generally higher proportion of children and teenagers who are serologically positive for rubella in South Yorkshire (resulting from high rates of infection in males seen in Fig. 3.6). These differences are seen not to be statistically significant.

The age-serological profiles, segregated by sex, for the S.E. England survey (Fig. 3.1 & 3.2) and for the survey of S. Yorkshire (Fig. 3.6) record a significant decline (up to one third of pre-vaccine levels) in the proportion of non-immune women of childbearing age.

The apparent reduction in immunity in males for

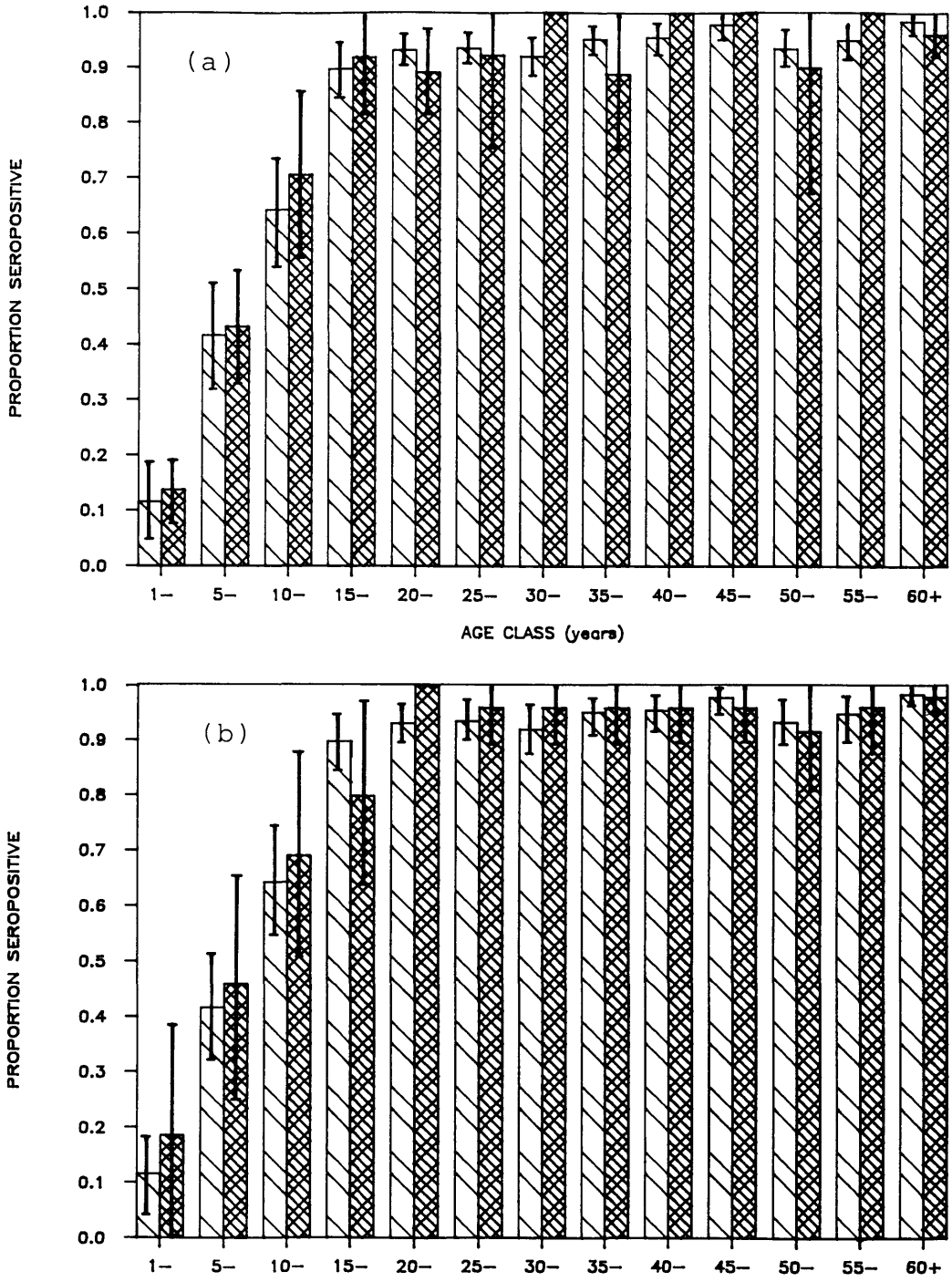


Fig.3.7 Rubella immunity in S.E. England 1980/84 compared with S. Yorkshire 1969 and 1985. The age-serological profiles for S.E. England 1980/84 (▨) is contrasted with that from S. Yorkshire (▩) for 1969 (upper graph) and 1985 (lower graph). The error bars illustrate 95% confidence limits. Sample sizes (pooled for males and females) are given in the legends to Figs. 3.1 & 3.5.

S. Yorkshire 1985, in the age range 10-19 years, compared with that shown for 1969, is not obvious in S.E. England 1980/84. It is therefore difficult to draw any conclusions about indirect consequences of female vaccination on male levels of seropositivity.

A small proportion of individuals (between 2% and 4%) remain susceptible in the upper age classes (60+ years) in both survey populations (Fig. 3.7a & b). Relatively large sample sizes give confidence in these results. It is possible that this may be an artefact of the serological technique, for instance, an inability of the RH test to detect antibody at low concentration or antibody which cannot mediate haemolysis, or the occurence of false negative results due to some blocking factor (Morgan-Capner, 1984). Alternatively, such a result could arise from, either, less than 100% exposure of the population to viral infection, or, genetically determined antibody production and persistence following infection (Anderson & May, 1985a).

The relatively low level of female immunity in the 50-59 year age range, compared with the general level of male and female immunity in the upper ages, observed for the S. E. England survey (Fig. 3.1), does appear to be reproduced in the S. Yorkshire 1985 results. The significance of these observations is not known.

3.4 Discussion.

Age- and sex-stratified serological profiles have produced

valuable information on the status of rubella immunity in S.E. England and S. Yorkshire, both pre- and post-vaccination. Results from these studies suggest that although total levels of immunity to rubella are unchanged since the initiation of vaccination policy, (compare S. Yorkshire 1969 with 1985), selective immunization of 10-15 year old schoolgirls has had a considerable impact on the serological status of young adult females. Between 5% and 10% of women 15-40 years old were found to be seronegative, depending on age, for both study areas.

Reductions in the proportion of non-immune females entering the 'at-risk' childbearing ages, similar to those which are reported here, have been described in other serological surveys. Ongoing studies by Clarke et al (1983) on North London blood donors and students from Nottingham University, confirm the divergence in the proportions of males and females immune to rubella. Of the women who had been eligible for school vaccination, the proportion seronegative varied beween 3% and 6% in different years, compared with 12-20% in men of the same age. From the same studies it was found that 2-6% of 15 year old Oxford schoolgirls (in 1979 and 1980) were non-immune, compared with 20-30% of males. In Leeds (Hambling, 1980) and Manchester (Tobin et al, 1985), the percentage of susceptible women attending neonatal clinics (7.7% for Leeds, 1978; 3.5% for Manchester, 1984), had slowly decreased during the last decade. The same situation existed among young women aged 15-19 years in Leeds (4.8% susceptible in 1978). Miller et al, 1985, reported 2.7%

antenatal patients and 8.3% non-pregnant women of the same age range, susceptible to rubella in the Manchester area, 1984. Furthermore, Public Health Laboratory reports to the Communicable Disease Surveillance Centre (CDSC), Colindale, of acquired rubella, suggest that the proportion of the total cases in pregnant women and in women of childbearing age have been steadily declining from 1978 through 1983 (Tobin et al, 1985).

The widespread effect of the nationwide selective immunization programme revealed in these serological surveys is encouraging. The result has been a steady decline in the annual incidence of congenital rubella syndrome (CRS) and of rubella associated terminations of pregnancy (Fig. 6.1).

Nevertheless, a significant proportion of females, of age 15-40 years, remain susceptible to rubella some 10-15 years after the start of vaccination. A number of reasons may account for this: (1) There has been insufficient time for the first immunized cohort of schoolgirls to age through the full range of 'at-risk' years. (2) This is compounded by the fact that vaccination rates have only been consistently high (> 80%) in the 1980s (see Fig. 6.11), and the benefit of these high rates will not be apparent for another 10-15 years. (3) Looking at the results of the S.E. England survey the policy of vaccinating seronegative women of childbearing age has not caused a discernable reduction in the pool of non-immune women too old to have been offered immunization at school (see Fig. 3.1 & 3.2, ages 30-39 years).

Undoubtedly the effort applied to implementing this

second stage of the policy varies across the country; dependent upon the enthusiasm of, and cooperation between, general practitioners, antenatal and postnatal clinics, virology laboratories, and follow-up workers (see Miller et al, 1985). A large survey of antenatal patients (> 20,000) from the Manchester area in 1984 (Miller et al, 1985), revealed a threefold difference in the susceptible proportion of nulliparous women (4.2%) and women in their second or subsequent pregnancy (1.4%). This disparity between nulliparous and parous women was statistically significant for the under twenties to the over thirties.

Spatial heterogeneity in vaccination levels (for both stages of the current policy) throughout the country, is one explanation for the differences in the proportions of seronegative women of childbearing age observed for the S.E. England and S. Yorkshire surveys, when compared with results from published surveys referred to above. How representative each study group is of the total population, is difficult to assess at present. A survey like that for the Manchester area by Miller et al (1985), has the advantage of size, but, surveillance of this nature is most likely to be undertaken in areas where health-care workers are conscientious with respect to vaccination objectives.

It is important to remember that the full impact of the selective vaccination policy will not be manifest at least until the first cohort of vaccinees has spanned the years in which females are fecund, and even after that time further reductions in CRS incidence might be expected as the most highly vaccinated cohorts progress through the years of

childbearing. This is unlikely to occur until the late 1990s, and consequently the decline in CRS to date (see Fig. 6.1) should continue for some years hence.

The proportion of schoolgirls vaccinated, by the age of 15, has plateaued over the last five years at about 85% (Fig. 6.11). It is, therefore, unlikely that the national target of 90-95% will be attained in the foreseeable future. Even under the impetus of the National Rubella Campaign (begun 1983), it is doubtful that adult female immunization will cover this shortfall. For the small, but significant, proportion of non-immune women of childbearing age that remain, a serious risk of infection during pregnancy still exists, due to continued circulation of wild virus in the community (particularly amongst males; note the decline in the proportion of seronegative males, over the age range 15-40 years, in Fig. 3.2). Consequently, it will not be possible to eliminate congenital rubella from the UK under the current programme of vaccination, and, therefore, there is a necessity to investigate vaccination policies which reduce the rate of transmission of rubella virus. The design and nature of such policies is the subject of Chapter 6.

Although the evidence from the studies reported here is inconclusive, serological observations by Clarke et al (1979, 1983) suggest that the proportions of susceptible schoolboys and young adult males in this country have increased slightly since the initiation of selective female vaccination.

A close similarity exists between the ASPs for rubella

in S.E. England and S. Yorkshire and those for rubella in other developed countries, (in the absence of mass vaccination), for instance, West Germany (Enders-Ruckle, 1969), Sweden (Svedmyr, Lundstrom & Thoren, 1967) and the United States (Kenny, 1976). A common feature is a 50-60% seropositive population by 10 years of age. The steepness of an age-serological profile indicates the magnitude of transmission of infection within a community (a concept which is quantitatively examined in the second part of this chapter). Differences in the rate of ascent of ASPs, with age, for a particular infectious disease can often be attributed to variation in social conditions or behaviour, such as the degree of mixing of individuals in urban or rural communities or between the sexes (see Anderson & May, 1983). However, it would be difficult to assign either of these causes to differences within and between the ASPs for S.E. England and S. Yorkshire (see Fig. 3.6 & 3.7).

Explaining the small proportion of individuals who remain susceptible to rubella infection in the oldest age classes (see Fig. 3.7) is important to the interpretation of age-related transmission rates and will be discussed in Part 2 of this chapter. This in turn influences predictions on the effectiveness of vaccination policies which are based on serological surveillance data (see Chapter 6). The possibility of genetic involvement in the determination of antibody status is investigated in Chapter 5, Part 2.

Part 2

AGE-RELATED VARIATION IN THE RATE OF TRANSMISSION

3.5 Introduction

The distribution of immunity to an infection in a population at one point in time may be ascertained from horizontal age-serological profiles. This was clearly illustrated for rubella in S.E. England and S. Yorkshire in the first part of this chapter.

Additionally, if it is assumed that horizontal changes reflect longitudinal trends, age-serological profiles, in the absence of mass vaccination, provide information on the rate of transmission of an infection in a population (Anderson & May, 1983, 1985a,b; Grenfell & Anderson, 1985). In general terms, the steepness of an age-serological profile (ASP) is a measure of the magnitude of transmission of the infection in the community: the more rapid the rise, the greater the degree of transmission (see Anderson & May, 1983, for examples).

More specifically, the changes, with age, in the proportion immune, are an indication of the rate at which susceptibles acquire infection. A quantitative measure of this infection rate is known as the 'force of infection' (denoted by the symbol λ) and may be defined as the

probability of a susceptible individual acquiring infection over a short period of time (i.e. an instantaneous per capita rate) (Anderson, 1982; Anderson & May, 1982a, 1983, 1985a,b; Grenfell & Anderson, 1985). Estimation of the force of infection, λ ,is made possible by the use of 'catalytic infection models' which mirror the changes in the proportion immune (i.e. proportion of the population who have experienced infection) recorded by age-serological profiles (or, alternatively, by age-prevalence profiles estimated from case notification data. See Chapter 4) (Muench, 1959; Griffiths, 1974; Anderson & May, 1982a, 1983, 1985a; Grenfell & Anderson, 1985).

It is worth pointing out that the force of infection is not equivalent to the more widely used 'attack rate'. The attack rate is defined as the number of cases in a specified stratification (e.g. age class) of the population over a given time interval, per head of population in that stratification (Grenfell & Anderson, 1985).

Simple observation of heterogeneity in the rate of contact between individuals within- and between-age classes in a community gives rise to the suspicion that the probability of a susceptible individual becoming infected (in an interval of time) is very much dependent on the age of that individual (for example, compare the frequency of interactions in school children with that between school children and say, old people). Indeed, age-dependence in the per capita rate of transmission has been documented for various childhood viral and bacterial infections, including measles, mumps, varicella, pertussis,

scarlet fever and rubella (Griffiths, 1974; Fine & Clarkson, 1982b; Anderson & May, 1985a; Grenfell & Anderson, 1985). A consistent age-related trend in the rate or force of infection has emerged from these studies: low in young children, high in older children and adolescents, and low again in adults.

Community based childhood immunization acts to increase the average age at infection above that which prevailed prior to control measures (evidence is both theoretical and empirical, see Knox, 1980; Fine & Clarkson, 1982b, 1983; Anderson, 1982; Anderson & May, 1982a, 1983; Bart et al, 1985a,b). For this reason the nature of age-dependent variation in the force of infection is thought to have considerable bearing on the outcome of mass vaccination programmes (Anderson & May, 1982a, 1983, 1985a, b; Schenzle, 1984; Grenfell & Anderson, 1985) particularly where the risk of serious complication arising from infection is itself age-dependent (Anderson & Grenfell, 1986; Anderson, Crombie & Grenfell, 1987).

Accurate determination of age-related rates of infection should, therefore, be regarded as a prerequisite for research involving the development of models which predict the impact of mass vaccination policy on disease incidence (Anderson & May, 1985a; Anderson & Grenfell, 1986).

Serological data is arguably the most reliable epidemiological information upon which to base such estimates (see Chapter 4 on the analysis of case notification data) (Anderson & May, 1983, 1985a). However, until now, there has been a distinct lack of detailed,

finely age-stratified serological data for rubella in the UK for this purpose. The surveys, described in the first part of this chapter, go some way to rectifying this situation.

In the following sections, age-related changes in the rate of rubella transmission are determined from the ASPs for S.E. England and S. Yorkshire, employing a 'polynomial catalytic infection model' described by Grenfell and Anderson (1985). The aim was to confirm the age-dependent nature of the force of infection for rubella, demonstrated in other studies, and to provide precise estimates of this parameter for a mathematical model of rubella virus transmission used to predict the incidence of CRS under various vaccination strategies (Anderson & Grenfell, 1986; Chapter 6). A discussion is given on the extent to which these results are representative of rubella in this country in the absence of mass vaccination, and on the factors which might affect confidence in the authenticity of estimates of infection rates from serological data.

3.6 A Polynomial Catalytic Infection Model

Age-specific forces or rates of infection, $\lambda(a)$, were estimated from age-stratified serological data for S.E. England and S. Yorkshire (detailed in Part 1 of this chapter) using the method described by Grenfell and Anderson (1985). In their approach, the rate of change, with age, in the proportion of the population immune to an infection, such as rubella, by age a, F(a), is represented by a

'catalytic infection model', which takes the form

$$\partial F(a) / \partial a = \lambda(a) [1 - F(a)] \qquad (1)$$

and has the solution

$$F(a) = 1 - \exp \left[- \int_0^a \lambda(\alpha) \partial \alpha \right] \qquad (2)$$

Previous models of this nature assume the force of infection, $\lambda(a)$, to be age-independent (Muench, 1957) or to increase linearly with age (Griffiths, 1974; Anderson & May, 1982a). Grenfell and Anderson (1985), however, represent the force of infection, $\lambda(a)$, as a polynomial function of age, a, and order k, where

$$\left.\begin{aligned} \lambda(a) &= \sum_{i=0}^{k} b_i a^i \qquad (D < a \leqslant U) \\ \lambda(a) &= 0 \qquad (A \leqslant D) \end{aligned}\right\} \qquad (3)$$

Here U denotes the upper age limit of the serological data employed to estimate the coefficients (the b_is) of the polynomial. The force of infection, $\lambda(a)$, is set at zero below the threshold D which represents the average duration of protection from infection, derived from maternal antibody (assumed to be 0.5 years; see Fig. 3.3).

Using equations (2) and (3) the b_i parameters of the $\lambda(a)$ polynomial can be estimated from observed changes, with age, in the proportion immune, F(a) (taken directly from age-serological profiles), by a method of maximum-likelihood

(Grenfell & Anderson, 1985)

The accuracy of this method in the determination of age-related forces of infection will be affected by the validity of assumptions incorporated into the model. These assumptions are: (i) the force of infection is constant with time (or at least exhibits constant recurrent cycles around an equilibrium level), (ii) the infection induces lifelong immunity to re-infection and does not increase the mortality of those infected, and (iii) horizontal age-related changes in the proportion of a population serologically positive mirror longitudinal changes for a cohort of individuals. For a stable population in which rubella is endemic the assumptions (i)-(iii) broadly hold (Grenfell & Anderson, 1985; Anderson & May, 1985a).

The mean age, A, at which individuals experience infection is also detemined from equations (2) and (3) (Anderson & May, 1983, 1985a, Grenfell & Anderson, 1985), where,

$$A = D + \int_0^\infty [1 - F(a)]\, da \qquad (4)$$

again D denotes the average duration of maternally derived antibody protection.

3.7 Force of Infection Estimates from Two Study Populations

(i) South East England

Fig. 3.8 and 3.9 display the results of applying the maximum-likelihood statistical procedure for estimating the age-dependent force of infection (Grenfell and Anderson, 1985) to male and female serological data for South East England 1980-84.

Fig. 3.8 records the change with age in the observed and expected proportion of males which have experienced infection with rubella (lower graph) and, the best fit polynomial model of age-related changes in the force of infection, λ (a) (upper graph). Average values for the force of infection over selected age intervals (0.5-5,5-10,10-15,15-20,20-40 years) are represented by the histogram bars. These facilitate clear description and easy comparison between results. The close correspondence between the observed age-prevalence profile and that expected from the estimated forces of infection (Fig. 3.8b) is indicative of the goodness of fit of the polynomial model (see Grenfell & Anderson, 1985).

Fig. 3.9 shows the corresponding results for females. Note that Fig. 3.9b records the change, with age, in the proportion seropositive as a result of natural infection and immunization. Fig. 3.9a, therefore, displays the polynomial function of the force of immunity (infection plus immunization) as a function of age.

Changes in the true force of infection for rubella (excluding the influence of vaccination), with age, may be obtained from the male data only (Fig. 3.8b). Under the current selective (female) vaccination policy in this country, it is assumed that transmission of virus to males

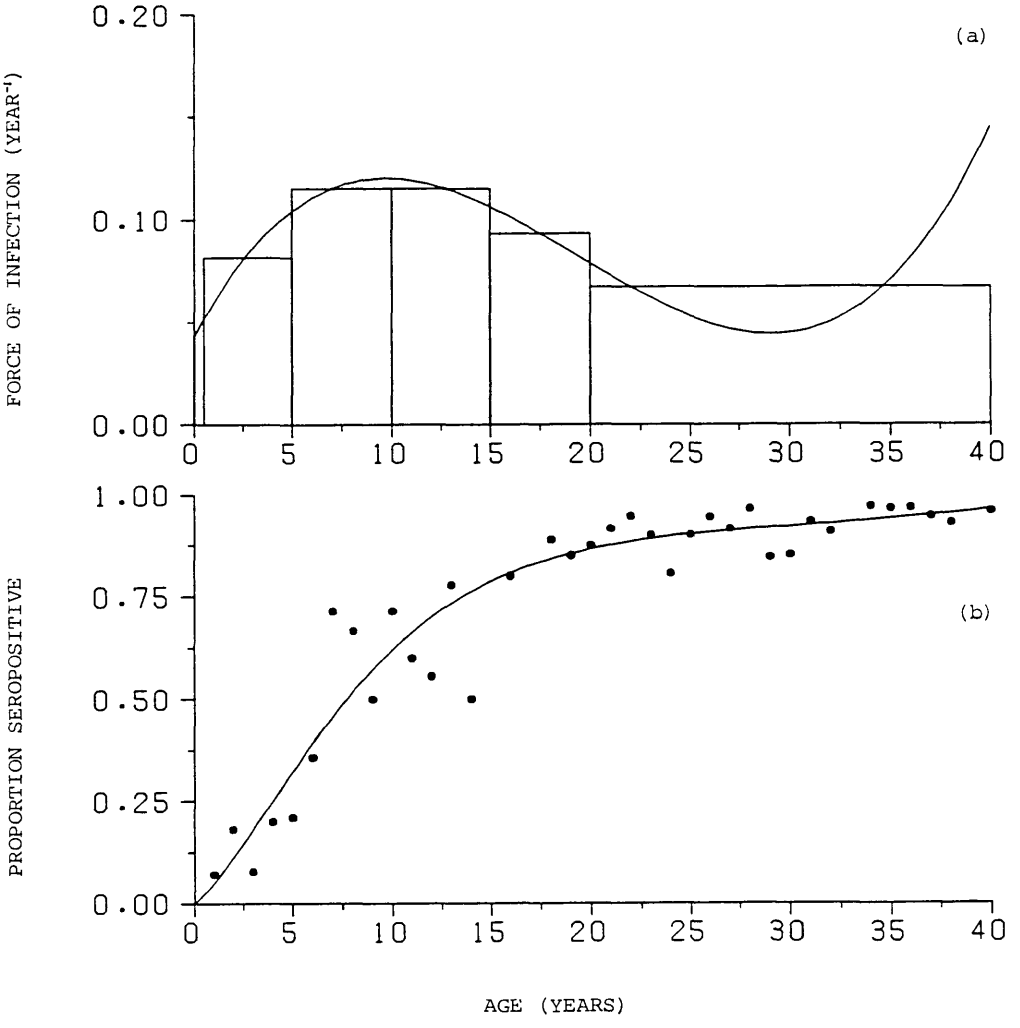


Fig.3.8 Estimates of age-dependent forces of infection for rubella derived from male serological data for S.E. England (1980/84). Graph (a) records the estimated values of the force of infection, $\lambda(a)$, derived by a method of maximum-likelihood (Grenfell & Anderson, 1985). Average $\lambda(a)$ values are represented for various age classes by the histogram bars. Graph (b) compares the predicted change, with age, in the proportion of males serologically positive (——) based on $\lambda(a)$ estimates defined in graph (a), with observed data (●).

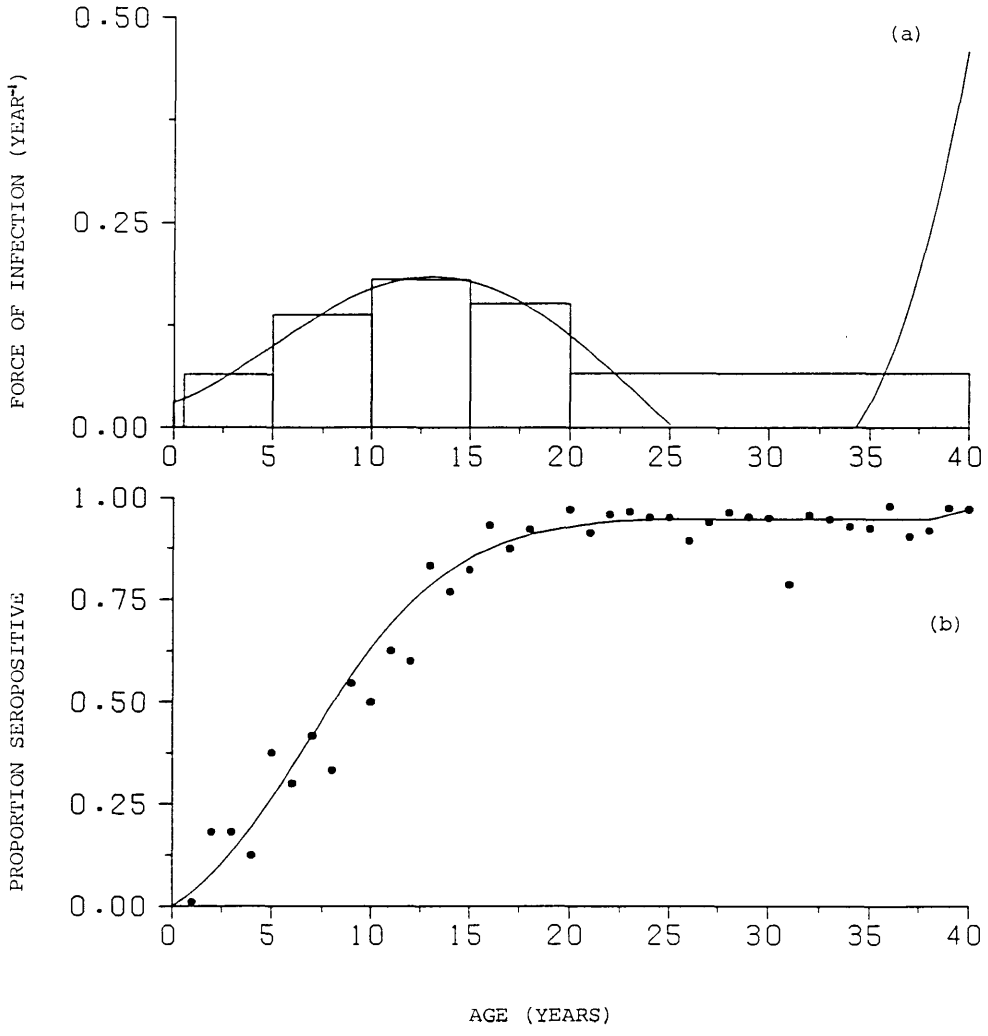


Fig.3.9 Age-dependent rate at which susceptible females acquire immunity to rubella (by infection and vaccination), estimated from serological data for females in S.E. England (1980/84). Graphs (a) and (b) are defined as in Fig. 3.8, except the observed data (●) are for females, therefore, the polynomial function shown in the upper graph and the predicted profile (——) in the lower graph reflect immunity in females resulting from both infection and vaccination.

is unaffected. This may be an over-simplification as a consequence of indirect effects (see later). The age-dependent rate of transmission for males (Fig. 3.8a) is seen to follow a convex trend; low in young children (age class 0.5-5 years), rising to a peak in older children and early teenagers (5-10 and 10-15 years old), followed by a decline to a relatively low value in the older age classes (20-40 years).

Estimates for $\lambda(a)$ (Fig. 3.8) for the ages 15-40 years may be assumed to reflect the rates of infection in females of childbearing age in the absence of vaccination.

A near twofold reduction in the probability of a male individual acquiring infection per unit time, is observed between the maximum force of infection (0.115/susceptible /year) in school children aged 5-15 years, and the minimum (0.067/sus/yr) in the 20-40 year age class (Table 3.2).

Close inspection of the observed female age-serological profile (ASP) (Fig. 3.9b) reveals a rapid rise in the proportion seropositive between the ages of 10 and 15 years, which is not seen in the male profile (Fig. 3.8b). This increased rate of acquisition of rubella antibody resulting from vaccination is reflected in the polynomial function of the force of immunity of Fig. 3.9a (which reaches a maximum over the same age range - note the scale, which is different from that for males in Fig. 3.8a), and subsequently in the steepness of the best fit profile (Fig. 3.9b). Comparison of the histograms in Fig. 3.8 & 3.9 (upper graphs) shows a peak value for the force of infection of 0.115/sus/yr for males (age range 5-15 years), whereas for females, what may

Table 3.2

Epidemiological characteristics derived from serological data pre- and post-onset of vaccination in the UK. (See text for analytical details).

Statistic	Seological Survey		
	S.E. England 1980/84		S. Yorkshire 1969
	Male	Female	Male
$\lambda(a)$ [1]			
0.5-5	0.0815	0.0647	0.0956
5-10	0.1155	0.1382	0.1244
10-15	0.1153	0.1805	0.1281
15-20	0.0932	0.1511	0.1154
20-40	0.0671	0.0667	0.0338
k [2]	3	4	2
A [3]	10.83		9.44
R_o [4]	7.3		8.4
T [5]	5.1		4.7
Fig. [6]	**3.8**	**3.9**	**3.10**

1. Force of infection estimates for different age classes
2. Order of $\lambda(a)$ polynomial function - see text;
3. Average age at infection estimated by a maximum-likelihood method (see Grenfell & Anderson, 1985);
4. Basic reproductive rate, where $R_o = L/A-D$; L is life expectancy (75 years) and D is the average duration of maternal antibodies (0.5 years) (see Anderson & May, 1985);
5. Interepidemic period, where $T = 2\pi(A.K)$; K is the sum of the latent and infectious periods (c. 22 days for rubella, Anderson & May, 1983);
6. Relevant figures for which statistics apply.

be termed the force of immunity, rises to 0.180/sus/yr in the 10-15 year age class (see Table 3.2).

The form of the polynomial function for females (Fig. 3.9a) between the ages of 15 and 40 is in response to a decline in the proportion of women immune to rubella in the mid- to late-twenties (returning to pre-vaccine levels), shown in Fig. 3.9b (and more clearly in Fig. 3.1 & 3.2). This marks the limit of influence of the teenage girl immunization policy; thereafter the proportion seropositive rises once more due to natural infection. Note that a negative value for the force of infection is not plausible in an epidemiological sense. The decline in female immunity is peculiar to horizontal age-stratified data (cross section of all ages at one point in time or over a short time interval), which would not have arisen if the serological survey had been longitudinal (following the infection of a cohort of individuals from birth through time and, therefore, age).

(ii) South Yorkshire

Fig. 3.10 displays the observed and expected age-serological profiles (lower graph) and the age-dependent forces of infection (upper graph) for the 1969 serological survey from S. Yorkshire.

Data for males and females have been totalled for the analysis. Using pooled samples it is possible to obtain a reasonable approximation to the overall pattern of pre-vaccination rubella transmission. This has been done in

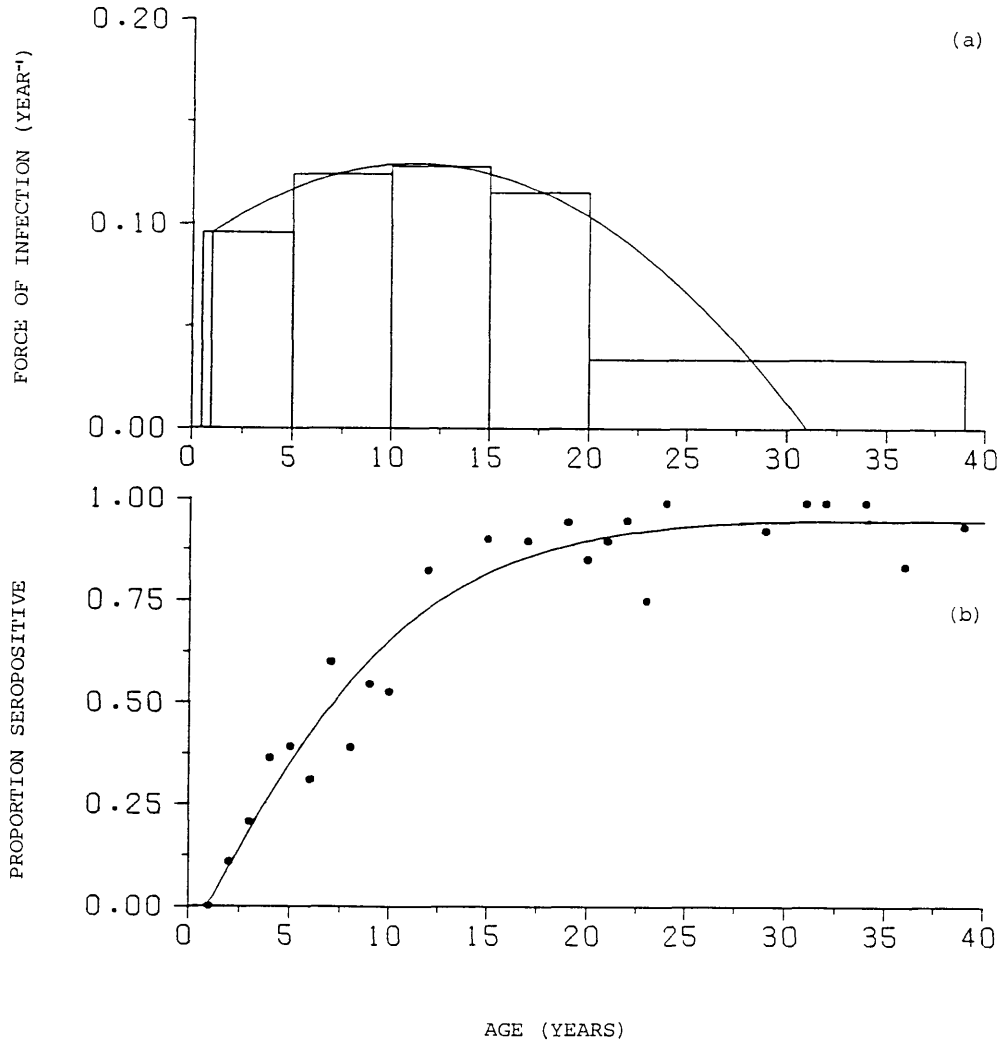


Fig.3.10 Estimation of age-dependent per capita rates of rubella infection for susceptibles in S. Yorkshire 1969. Graphs (a) and (b) are as for Fig. 3.8 except that the raw data (●) come from the 1969 survey of S. Yorkshire (male and female totals).

spite of differences observed between male and female profiles (see Part 1 of this chapter), but for whom individual sample sizes were relatively small.

Only the 1969 survey from S. Yorkshire has been analysed for age-dependent transmission rates, because it is unaffected by rubella vaccination. As such, it represents a control against which to compare the results derived from the S.E. England male serology (Fig. 3.8). Segregation of male results for the 1985 S. Yorkshire survey was posssible but large fluctuations in prevalence (see Fig. 3.6a), with age, prevented a critical analysis.

Results of applying the maximum-liklihood method to the 1969 ASP (Fig. 3.10b) reveal a marked age-dependence in the force of infection (Fig. 3.10a) which is convex in nature. The transmission rate changes from low (0.5-5 year age class), to high (5-15 year olds), to low again (20-39 years; the upper age is determined by the empirical data).

This trend in $\lambda(a)$ illustrated in Fig. 3.10a bears a close resemblance to that shown by the male serology from S.E. England (Fig. 3.8a). Quantitative agreement is, in general, good (Table 3.2). Maximum estimates of $\lambda(a)$, for both surveys, are exhibited by children of school age (5-15 years); specifically, 0.115/sus/yr for 1980/84 male serology and 0.128/sus/yr for 1969 total serology.

The predicted average age at infection (which assumes a duration of maternally-derived immunity of 6 months, see Fig. 3.3) for pre-vaccine males and females (1969) is 9.4 years. For males (1980/84), 10-14 years post onset of vaccination, a somewhat higher value of 10.8 years is

estimated. The diference between the two estimates results from slightly higher infection rates for the South Yorkshire survey, shown in Fig. 3.10a.

By comparing the histogram bars in the upper graphs of Fig. 3.8a & 3.10a it can be seen that the rate of infection in adults (20+ age class) from S. Yorkshire (0.034/sus/yr) is approximately half that for males of the same age from S.E. England (0.067/sus/yr) (Table 3.2). Whereas for the male serology of S.E. England a twofold decline from maximum $\lambda(a)$ in children to the minimum in adults is recorded, a fourfold decrease in $\lambda(a)$ is estimated for the pre-vaccination S. Yorkshire data.

3.8 Discussion

An important feature of the results of this section is the nature of the age-related change in the force of infection shown for data from S.E. England (males) and S. Yorkshire (totals). In each case, transmission rates are lowest in young children (0.5-5 years old) and adults (20+ years of age), and highest in older children and teenagers (5-15 years old).

Similar trends have been recorded for other childhood viral infections such as measles, mumps and chickenpox (Fine & Clarkson, 1982b; Anderson and May, 1985a; Anderson, Crombie and Grenfell, 1987). Maximum rates of infection invariably occur within the age range 5-15 years, corresponding to the years of mass school attendance. The

high degree of physical contact of susceptible and infectious individuals throughout the school years most probably accounts for these observations. Support for this explanation comes from early reports (Hamer, 1906; Soper, 1929) of the close association between weekly case notifications for measles, with seasonal cycles of school term and vacation time (see Anderson, Grenfell & May, 1984, and Fine & Clarkson, 1982a, for recent analyses).

Immunity resulting from natural infection or from rubella immunization is not distinguished in the results for females, which, therefore, record the force of immunity in the age ranges where vaccination is offered (most importantly the 10-15 year olds). Consequently, only data for males from the S.E. England survey are likely to mirror pre-vaccine transmission rates. Indeed, there is good general agreement between values of $\lambda(a)$ for males in 1980/84 and those for the total S. Yorkshire data (see Table 3.2).

Estimates for the average age at infection (low in 1969, high in males 1980/84) reflect the earlier acquisition of rubella antibodies (particularly males aged <10 years) in S. Yorkshire compared with S.E. England, and, perhaps, a reduction in the net force of infection in the community due to female vaccination. However, the indirect effect of female vaccination on rubella infection in males is unlikely to be of great significance because of the age at which vaccine is administered. On the other hand, it is possible, that differences in social behaviour between these areas of the country largely account for the dissimilar average ages

at attack (if so, results of a similar nature should be obtained from surveys of other childhood viral infections).

Age-related changes in the rate of transmission have important implications to the design of vaccination programmes, such as estimation of the critical proportion of the population requiring vaccination for eradication (Anderson & May, 1985a,b). For rubella, where the risk of serious disease arising from infection is so distinctly age-dependent, the reduced force of infection in adults recorded for the surveys reported here, is of considerable applied significance (Anderson & Grenfell, 1986). Under mass vaccination, during which there is a tendency for the average age at infection to increase (Knox, 1980; Fine & Clarkson, 1982b, 1983; Anderson, 1982), the changes observed in $\lambda(a)$ may result in susceptible individuals experiencing lower rates of infection from those to which they would have been exposed in the absence of immunization (Grenfell & Anderson, 1985; Anderson & May, 1985a). What effect this is predicted to have on the incidence of rubella in women of childbearing age and, therefore, on the number of cases of CRS, is considered in Chapter 6 (see also Anderson & Grenfell, 1986).

The rate of infection in adults in this country, in the absence of mass vaccination, is probably most accurately reflected by that estimated for males from the S.E. England survey (when compared with the result from S. Yorkshire data, for which sample sizes dwindle in the older ages). However, the effect of the vaccination programme on this result is difficult to quantify.

The relatively low infection rates recorded in the older age classes for both study areas may have a number of explanations, for example, heterogeneity, with age, in mixing or in infectiousness, or insensitivity of the serological technique, or genetic or social inhomogeneity (see Chapter 5, also Anderson & May, 1983a; Grenfell & Anderson, 1985). The validity of age-related changes in the force of infection estimated from serological data is, in part, dependent upon which of these possible generative processes is dominant (Anderson & May, 1985a). This issue is returned to in Chapter 6 in connection with mathematical model predictions of the incidence of congenital rubella.

Chapter 4

AGE-STRATIFIED CASE NOTIFICATIONS: THE ALTERNATIVE TO SEROLOGICAL DATA

4.1 Introduction

Public health records of infectious disease notifications are another source of valuable epidemiological information in this country which, in the past, have been more commonly utilized than serological surveys (Anderson & May, 1983a). Case notifications for diseases such as measles, mumps, pertussis and rubella, over a specified time interval (a month, a year, an epidemic cycle), for which reports are stratified by age class, yield information on the change, with age, in the proportion of a community or a population who have experienced a particular viral or bacterial infection (Griffiths, 1974, Fine & Clarkson, 1982b, Grenfell & Anderson, 1985, Anderson & May, 1985a). These age-related changes in the proportions immune provide a basis for the estimation of age-dependent rates of infection in the community, as discussed in Part 2 of Chapter 3 (Anderson & May, 1982a; Grenfell & Anderson, 1985).

Age- and sex-related trends in rubella case notifications obtained from various sources in England are

explored in this chapter. There are two aims of the study. The first is the provision of epidemiological data of a type different from that derived from serological surveillance which was presented in Part 2 of the preceding chapter. The purpose is to determine if there are consistencies in important characteristics such as the age-related nature of rubella transmission rates and the impact of vaccination on virus transmission, between the two types of information,which would give added assurance that the results were reliable.

The second aim is to justify the need for rubella epidemiological data based on serology by establishing that (i) there is a paucity of the only alternative data source in this country, and (ii) there are serious limitations in the assumptions underlying the analysis of case notifications.

Accuracy in the estimation of empirical parameters, such as age-specific forces of infection, is a prerequisite to accurate forecasting of the impact of vaccination programmes using mathematical models of virus transmission (Fine & Clarkson, 1983). This study is intended to review the merits of information derived from case notifications to allow a critical evaluation of model predictions based on rubella notifications or serological data in Chapter 6.

4.2 Sources of Case Reports

Rubella is not nationally notifiable in the UK in contrast,

for example, to measles, whooping cough and diptheria (source of data: OPCS). However, there are five cities in England (namely, Exeter, Bristol, Leeds, Manchester and Newcastle) for which notification by their respective health authorities is requested (Tobin et al, 1985). Furthermore, numbers of confirmed infections by Public Health Laboratories and cases clinically diagnosed by members of a group of practices selected by the Royal College of General Practitioners (RCGP) Research Unit in Birmingham, are collated by the Communicable Disease Surveillance Centre (CDSC), Colindale, London.

Unfortunately, data that are finely divided by age, and sex stratified, are available from only two sources, Bristol (supplied by Frenchay Health Authority) and Leeds (supplied by Leeds Eastern Health Authority). Pre-vaccination data for Bristol for the years 1967-1969, and notifications post onset of immunization for Leeds (1978) and Bristol (1978-83) have been obtained and are used in the analyses.

4.3 Method of Analysis and Underlying Assumptions

The accumulated proportion of cases, with age, from notifications reported over a short time interval (such as those for Bristol and Leeds referred to above), mirrors the cumulative infection of a cohort of individuals from birth through time. Recovery from many acute viral infections (like rubella) confers durable immunity to re-infection, and as a consequence these data represent age-related

changes in the proportion of the population who have specific immunity.

Implicit in this assumption is that the rate of transmission for the infectious disease agent is constant through time. Seasonal fluctuations in rates of infection are well known (see Fine & Clarkson, 1982a). Infection rates also vary over epidemic cycles and even longer periods (Grenfell & Anderson, 1985). Nevertheless, the average value will remain relatively constant with time in the absence of control measures (i.e. mass vaccination) if notifications span at least a year, or an epidemic cycle.

The supposition that horizontal age-stratified information reflects longitudinal trends applies equally to serological data as it does for case reports. However, for case notifications there is the further assumption that every age group is reported with equal efficiency (Collins, 1929; Fine & Clarkson, 1982b), i.e. there is no age-related bias in reporting. In practice, for many childhood viral infections there is thought to be a tendency to under-report cases in upper age classes (Evans (ed.), 1982; Belshe (ed.), 1984). This may be confounded in the case of rubella (for which the dangers of infection during pregnancy are widely known) by more accurate observations in females of older age, which has been referred to as the 'Gregg effect' (Ingalls et al, 1960).

A further implication of using notifications to represent the cumulative proportion of individuals infected as age increases (also known as an age-prevalence profile), is that inevitably 100% of the population is infected by the

upper age of the reported cases. Such an assumption is open to doubt particularly in the light of serological evidence to the contrary (see Chapter 3, Part 1). This will be discussed at the end of this section.

Bearing in mind these limitations, an age-prevalence profile (APP) constructed from rubella notifications provides some information on the rate of transmission of infection within the community. Just as with age-serological profiles, changes with age in the force of infection may be determined using the maximum-likelihood method described by Grenfell and Anderson (1985) (see Chapter 3, Part 2).

4.4 Analysis of Case Reports from Two English Cities

(i) Pre-vaccination data

Trends in rubella reports from the city of Bristol between the years of 1967 and 1969 are presented in three ways (Fig. 4.1a-c). Fig. 4.1a records the frequency of reported cases stratified by age. The numbers in each age class for each year are pooled over the three years. This irons out inconsistencies arising from changing transmission rates or from variable reporting (Fine & Clarkson, 1982b). Fig. 4.1b depicts the cumulative proportion of cases with age, and Fig. 4.1c the age-related changes in the force of infection. Results for each sex are shown separately. The preponderance of reported cases in the young age classes (0-14 years) is

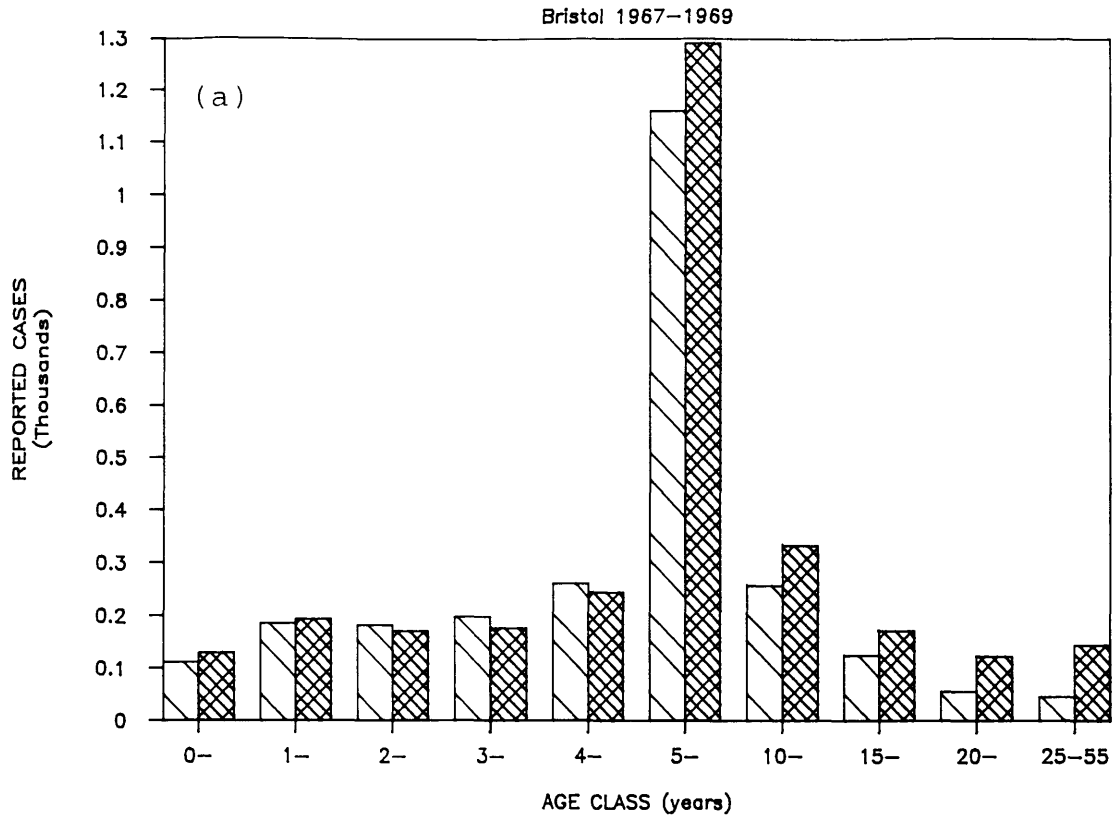
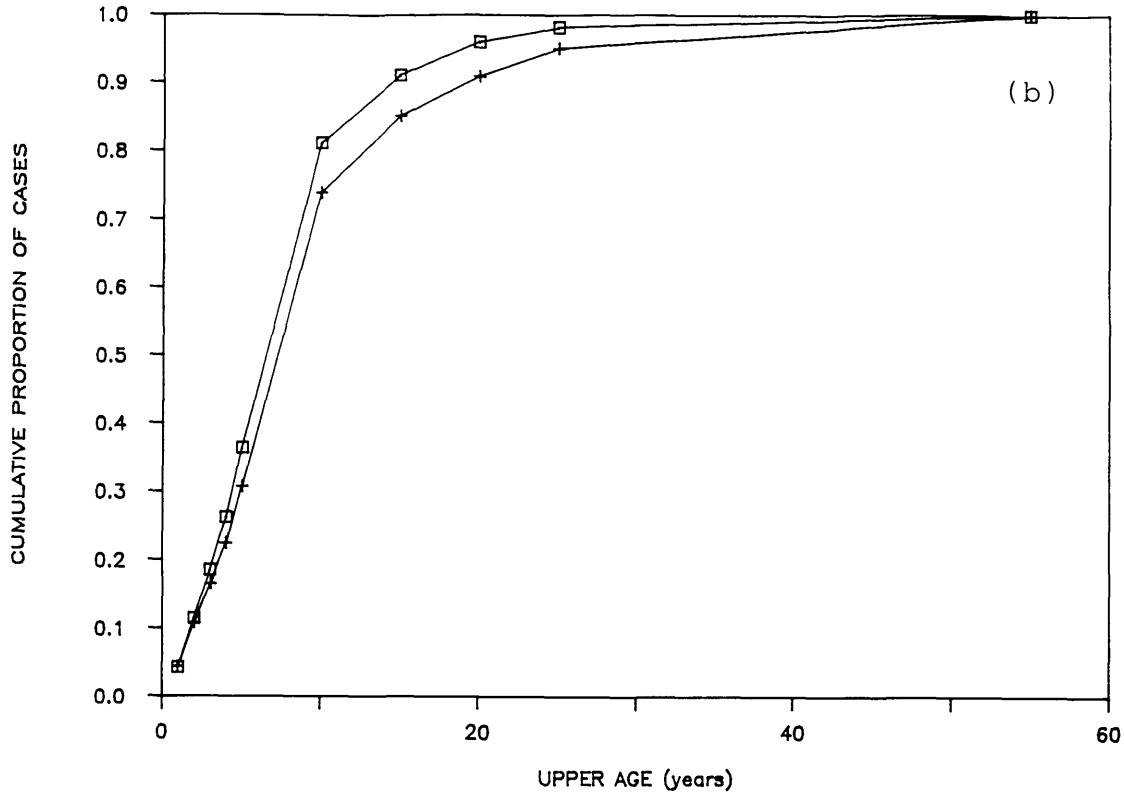
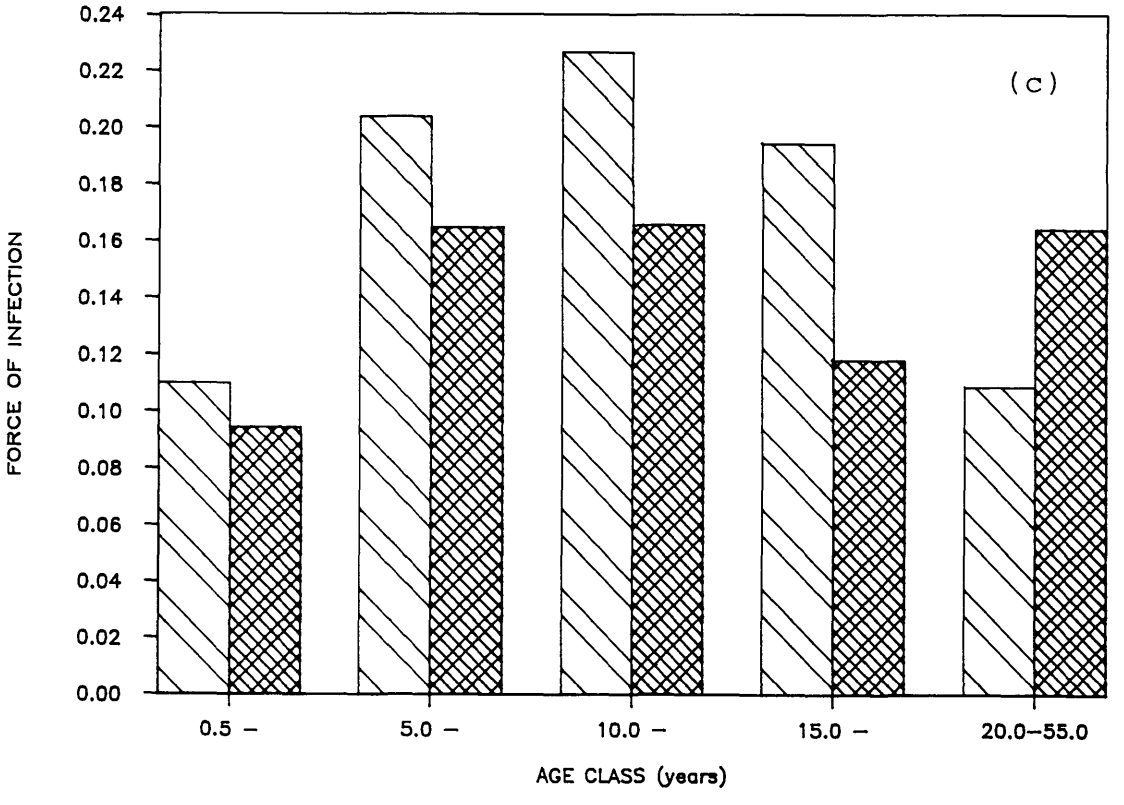


Fig.4.1 Reported cases of rubella from the city of Bristol between 1967 and 1969 (data source: Frenchay Health Authority, Bristol). Graph (a) records the total frequency of cases during the period 1967-69 stratified by age, showing males (▨) and females (▩) separately. The frequencies in graph (a) are accumulated with age to produce age-prevalence profiles for males (□—□) and for females (+—+) and shown in graph (b). Graph (c) records the age-specific forces of infection, $\lambda(a)$ (calculated by the method of Grenfell & Anderson, 1985), for each sex (identified as for graph a), derived from the cumulative proportions of cases shown in graph (b).


(b)
CUMULATIVE PROPORTION OF CASES
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
20
40
60
UPPER AGE (years)


(c)
FORCE OF INFECTION
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.5 –
5.0 –
10.0 –
15.0 –
20.0–55.0
AGE CLASS (years)

characteristic of any childhood viral or bacterial infection (Ingalls et al, 1960; Witte et al, 1969; Brown & Wilson (eds.), 1984).

Rubella reports for males and females (Fig. 4.1a) are similar in young children (0-4 years), but upwards from the 5-9 year age class there is increasing divergence in numbers reported, to the extent that three times more cases are reported for females over the age of 25 years than for males of the same age.

As a result of the high incidence of reports in the upper years, the cumulative proportion of cases for females lags behind that of males (Fig. 4.1b). Consequently, consistently higher age-dependent rates of transmission for males, compared with females, in all age classes except the eldest (20-55 years), are recorded as seen in Fig. 4.1c.

It is probable that the differences in case reporting efficiency between the sexes (Fig. 4.1a), and the subsequent discrepancies shown in Fig. 4.1b & c, result from greater awareness, by the general public and general practitioners, of the risk associated with German measles in pregnant women. Without knowledge of the reporting bias recorded in Fig. 4.1a the obvious, and possibly fallacious, conclusion from the results in Fig. 4.1b & c would be that infection with rubella in males occurs, on average, at a younger age than in females. These results are, therefore, not a straightforward confirmation of the differences in transmission rate between the sexes suggested by the serological data for S. Yorkshire (Fig. 3.6). They appear to arise as a consequence of biases in the efficiency of

case reporting.

A marked age dependence in the force of infection for this pre-vaccination rubella data is shown in Fig. 4.1c. Despite inconsistencies in reporting between the sexes, a general trend to vary, as age increases, from relatively low, to high, to low transmission rates, is seen for both males and females (see Table 4.1).

The same convex pattern was noted for serological data (see Part 1 of Chapter 3). However, the magnitude of the $\lambda(a)$ values for the case notifications, generally exceed those derived from the age-serological profiles for S.E. England (male serology Fig. 3.8) and S. Yorkshire (total Fig. 3.10). Note how the high rates of infection derived for the Bristol pre-vaccine data (particularly in schoolchildren) are reflected in low estimates for the average age at infection for males and females, when compared with serologically determined results (compare Table 3.2 with 4.1). High transmission rates, and corresponding low estimates for the average age at infection, derived from notifications for Bristol, might reflect a widely held belief that the probability of reporting a case of a childhood viral or bacterial infection for either sex in the younger age-classes, is higher than that for adults (Collins, 1929; Fine & Clarkson, 1982b).

(ii) Post-vaccination data

The frequency of reported cases of rubella, by age class and sex, in Leeds for 1978, and in Bristol for the years 1978

Table 4.1

Epidemiological characteristics derived from rubella case notifications pre- and post-onset of vaccination in the UK. (For analytical details see text and parameters as defined for Table 3.2).

Parameter	Source of Notifications						
	Bristol					Leeds	
	1967-69			1978-83		1978	
$\lambda(a)$	Male	Female	Total	Male	Female	Male	Female
0.5-5	0.1104	0.0950	0.1037	0.0783	0.0833	0.0895	0.1016
5-10	0.2043	0.1653	0.1849	0.1401	0.1448	0.1337	0.1379
10-15	0.2272	0.1663	0.1872	0.1596	0.1486	0.1516	0.0946
15-20	0.1949	0.1187	0.1339	0.1487	0.1141	0.1490	0.0614
20+	0.1092	0.1647	0.1753	0.1436	0.0977	0.1259	0.1379
k	3	3	3	3	3	3	4
A	7.22	8.77	8.04	9.22	9.72	9.03	9.62
R_0	11.2	9.1	9.9	8.6	8.1	8.8	8.2
T	4.1	4.6	4.4	4.7	4.8	4.6	4.8
Fig.	**4.1**	**4.1**	**6.2**	**4.3**	**4.3**	**4.4**	**4.4**

through 1983, are illustrated in Fig. 4.2a & b respectively. It is encouraging to note the degree of resemblance in the pattern of reporting in these two cities. This gives some assurance of their representation of countrywide patterns.

Interesting observations arise from comparing these post-vaccination notifications (Fig. 4.2a & b) with those for pre-vaccine years (1967-69) (Fig. 4.1a). Considerable qualitative similarity in the overall trend in reporting, with age, is seen between each histogram. However, the bias towards reporting of cases in females, compared with males, for the age classes 5-9 to 25-55 years, which was evident in pre-vaccination data (Fig. 4.1a), is reversed in the reports for age classes 10-14 to 15-19 years for Leeds 1978, and for age classes 10-14 to 20-24 years for Bristol 1978-83 (Fig. 4.2).

The inference is that for these particular age ranges, in Fig. 4.2a & b, the proportion of susceptible females in the population and, subsequently, the incidence of infection, has been reduced by vaccination of 10-15 year old girls. The span of ages influenced is dependent upon the number of years post-initiation of vaccination (1970) for which reports are obtained. This observation helps explain the difference in results between the 1978 Leeds and 1978-83 Bristol notifications.

An equivalence in the frequency of male and female case reports for those aged less than 10 years, and a reporting bias between the sexes (female exceeding male) in those too old to be influenced by immunization, are two features to be found in post-vaccination figures that were also present for

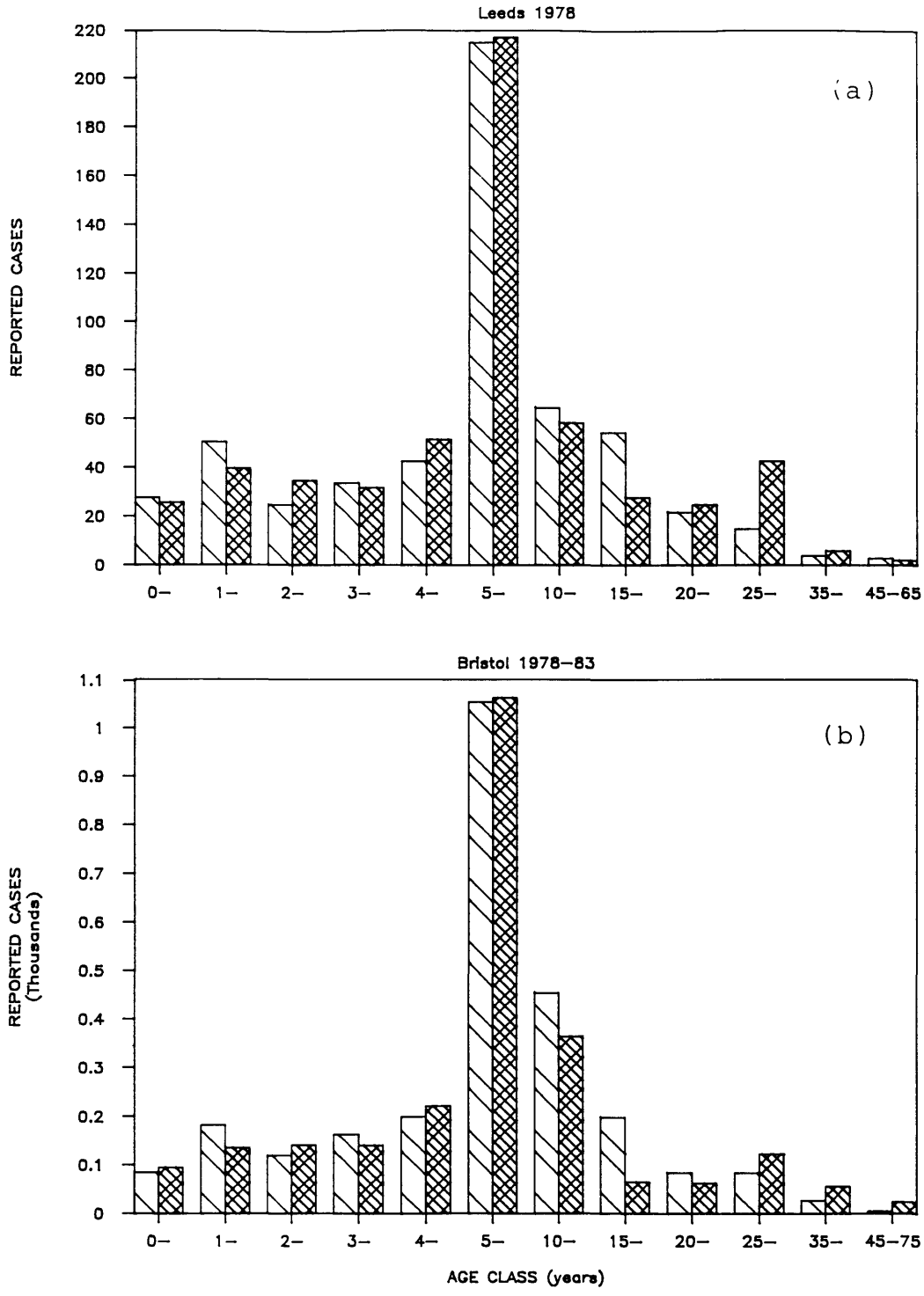


Fig.4.2 Reported cases of rubella post-onset of vaccination, for two cities in England. Frequency of cases stratified by age and sex (▨ , males; ▩ , females) from, (a), Leeds 1978 (source: Community Health Services, Leeds Eastern Health Authority) and, (b), Bristol 1978-83 (source as in Fig. 4.1).

pre-vaccine data. This is to be expected for an immunization programme which is selective in nature.

Age-prevalence profiles and resultant age-related changes in the force of infection (maximum-likelihood method), are derived from the case reports shown in Fig. 4.2a & b, and recorded, for males and females separately, in Fig. 4.3 & 4.4.

The cumulative proportion of cases for males from Leeds and from Bristol shown in Fig. 4.3a & 4.4a respectively, give rise to the now familiar convex age-dependent relationship for the force of infection seen in Fig. 4.3b & 4.4b. However, considerably less decline is observed in $\lambda(a)$ from the maximum estimate in the 10-15 year olds, into the upper ages (20+ years), than was exhibited by serological data (Fig. 3.8 & 3.10), or male pre-vaccination notification results (Fig. 4.1c). Furthermore, the $\lambda(a)$ estimates in Fig. 4.3b & 4.4b, though much in excess of those described for serology (see Table 3.2), are less than the estimates obtained from pre-vaccination records (Table 4.1) in all age classes below 20 years.

These differences between pre- and post-vaccination infection rates for males are consistent with a higher proportion of cases occurring in the older age classes ($\geqslant$20 years in Fig. 4.3b & 4.4b). This may result from fewer male cases of infection in those age classes with a high degree of interaction with vaccinated female cohorts (particularly 10-15 and 15-20 year age groups), hence the lower $\lambda(a)$ values in these classes shown in Figs. 4.3b & 4.4b compared with Fig. 4.1c.

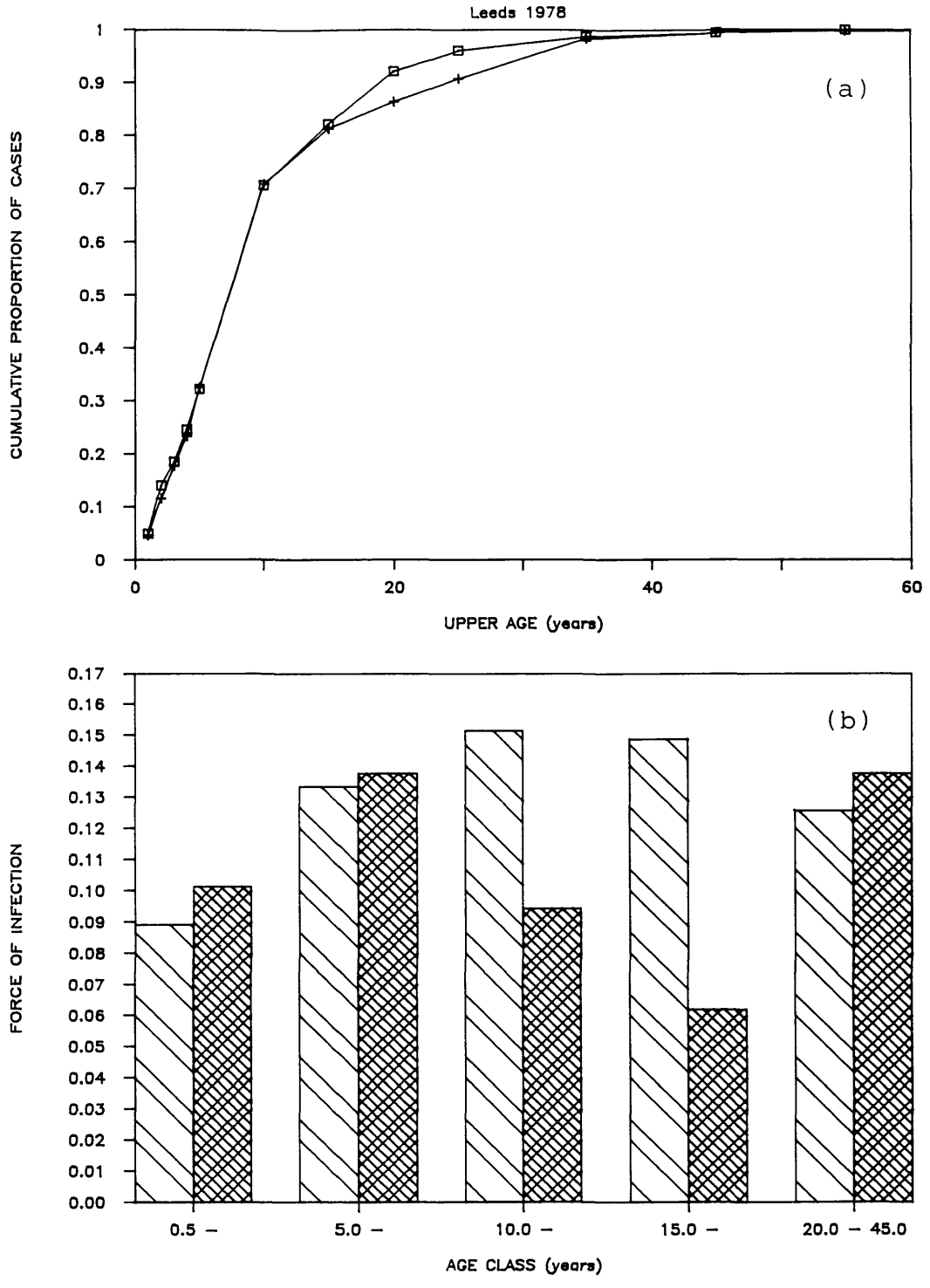


Fig.4.3 Analysis of case notifications for Leeds in 1978. Graph (a) records the cumulative proportion of cases, with age, for males (□—□) and for females (+——+). Age-specific forces of infection, $\lambda(a)$, are calculated as in Fig. 4.1, for males (▨) and females (▩), and recorded in graph (b).

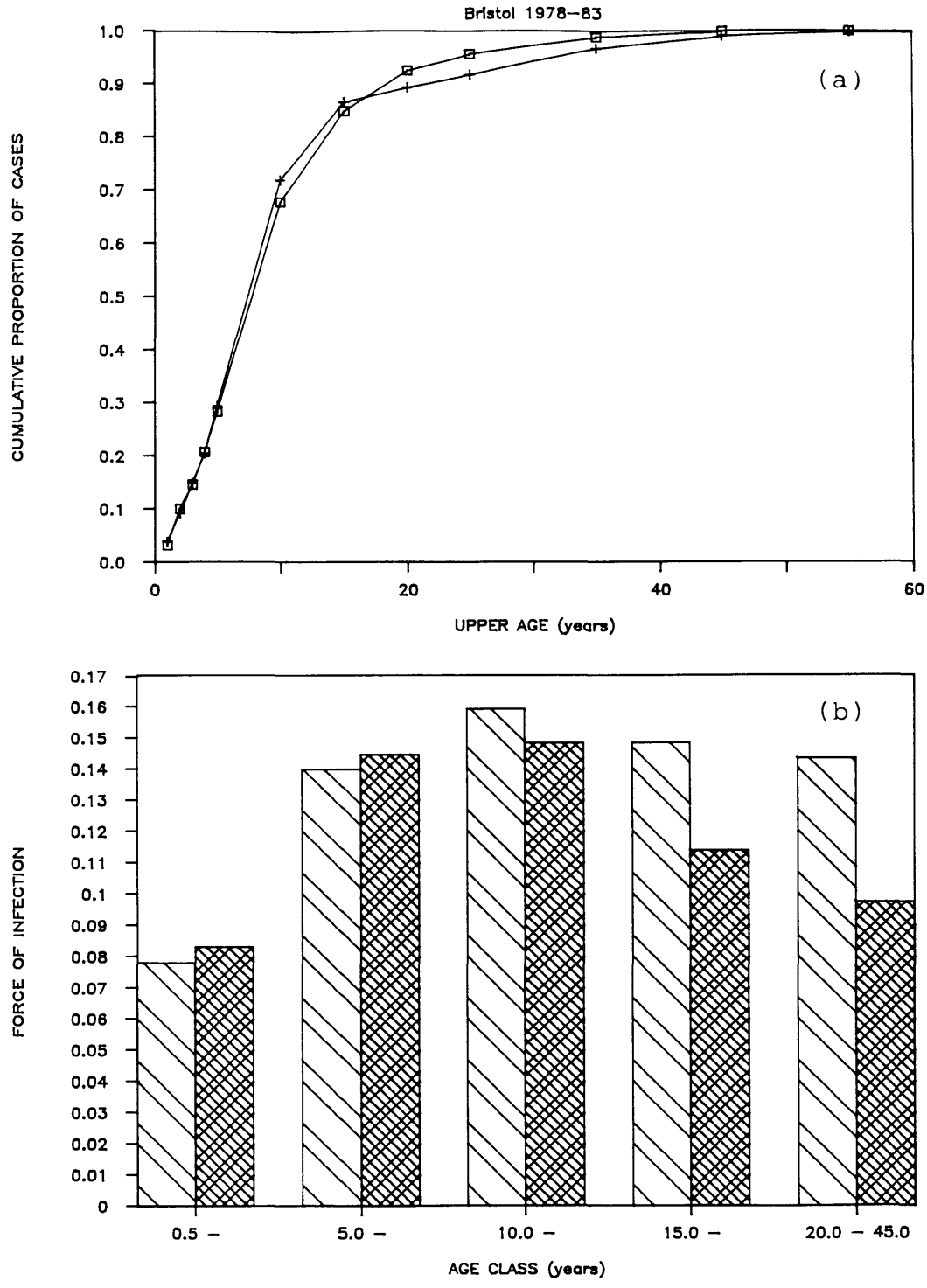


Fig.4.4 Analysis of case notifications for Bristol over the period 1978-1983. Other details are as shown in Fig. 4.3.

Comparing the APP for males and females from pre- and post-vaccination data, the lag in the cumulative proportion of female cases prior to immunization (Fig. 4.1c) arising from age-dependent bias in female reports, is counteracted, in post-vaccination results (Fig. 4.3a & 4.4a), by the lower incidence of female cases (seen in Fig. 4.2a & b) in those age classes affected by the schoolgirl immunization programme. This results in proportionally more cases occuring in the younger age range than was observed prior to vaccination.

The distinctly lower rate of increase in the cumulative prevalence profiles for females, compared with males, above 15 years of age in Fig. 4.3a & 4.4a, and the consequent reduction in the rate of transmission in females in Fig. 4.3b & 4.4b, results from the relatively low frequency of female cases of infection in the 10-19 year olds for Leeds (Fig. 4.2a) and 10-24 year olds for Bristol (Fig. 4.2b) under the influence of vaccination. The low $\lambda(a)$ estimates are a reflection of less cases of rubella in females in the community and do not necessarily represent a reduced probability of a susceptible individual acquiring infection (although the net force of infection may decline as a result of fewer infectious females in age classes covered by vaccination).

Convergence of male and female APPs in the adult age classes occurs where vaccination has negligible impact (note that this takes place at an earlier age in Fig. 4.3a, for Leeds, because the reports are less recent than those for Bristol, Fig. 4.4a). This results from the bias towards

reporting of females which was seen in Fig. 4.2a & b.

4.5 Discussion

The cumulative proportion of rubella cases (notified over a short time interval), with increasing age, reflects time-dependent changes in the proportion of a cohort of individuals who have experienced infection. Under this premiss, age-prevalence profiles constructed from rubella notifications for Bristol (1967-69 and 1978-83) and Leeds (1978), have been analysed for age-related changes in the force of infection (per capita rate at which susceptibles acquire infection) using the polynomial catalytic infection model described by Grenfell & Anderson (1985) (see Part 2, Chapter 3).

Results shown in this section, for pre- and post-vaccination data reinforce the impression of a consistent pattern of change in infection rates, with age, which was evident in rubella serological data (see Table 3.2) and in analyses of other childhood viral and bacterial infections in developed countries (Anderson & May, 1985a, b).

In general, maximum rates of transmission are observed in children and young teenagers, between the ages of 5 and 15 years. The major generative factor is probably the high degree of interaction of susceptible and infectious individuals whilst attending school (refer to section 3.8 for more discussion).

Two important points have come to light from the analyses in this section which are of considerable concern to interpretation of the results derived from case notifications. The first relates to the reliability of the empirical data. Similarity in the results for Leeds and for Bristol give some assurance that the reporting characteristics of these two cities are likely to be broadly representative of the country. Nevertheless, data acquired on a national basis (were it available for the UK) would provide a more reliable database for analysis. Of more importance, however, is that notifications from both sources (pre- and post-vaccination) suffer from age-dependent bias within and between the sexes.

Generally speaking, the force of infection estimates derived for case notification data in this section are higher than those described from serological sources in Chapter 3. This probably arises from under-reporting of cases in older age classes which results in a higher proportion of infections occuring in younger ages and a consequent low average age at attack. This view, although generally held to be true, needs to be contrasted with evidence which suggests that as many as 50% of childhood rubella infections are subclinical (Banatvala & Best, 1984) and that the severity of disease from infection tends to increase with age (Lamprecht, 1984).

Grenfell & Anderson (1985) point out that, in principle, case reports should be weighted according to the number of people in each age class within the total population. Predictions for the United Kingdom (OPCS, PP2 No.8) reveal a

relatively uniform age distribution over the age range 0-44 years, but the proportion of the population in each age class declines thereafter with increasing age. Biased reporting in the upper ages may result from this demographic characteristic.

There is clear indication of a bias towards over-reporting of female cases of rubella infection, compared with males, in all age classes other than those of childhood. This is assumed to be the manifestation of an awareness (e.g. by parents with daughters, women of childbearing age and Doctors) of the risk of CRS from rubella during pregnancy, in females who have not previously experienced infection.

Disproportionately high reporting for females, compared with males, particularly in the adult age classes, accounts for the relatively low transmission rates for females obtained from pre-vaccination data. This leads to the mistaken conclusion that male infection occurs, on average, at an earlier age than in females. Immunization of schoolgirls against rubella has significantly reduced the proportion of cases that occur in females, in post-vaccination data, over the age range now embraced by the policy. However, the impact on female cases is somewhat masked in the results, particularly when compared with male reports, because of the underlying bias towards female notification.

The second point of concern arises from the procedure whereby APPs are constructed from case notifications (i.e. by accumulating the proportion of cases with age). One

fault with this method is that factors which modify case reports for particular age classes (i.e. an influence which is non-uniform over all ages) will have an inverse effect on the proportion of cases in all other age groups not directly influenced. Two such factors observed in the results of this section were age-dependent bias in reporting for males and females, and selective female vaccination, which could lead to misinterpretation of the calculated age-related transmission rates.

Another fault of the APPs produced from case reports (one which has applied significance) is the presumption that all individuals experience infection at some point in their lifespan. The indication from published serological surveys of rubella (Nokes, Anderson & Anderson, 1986), mumps (Wagenwoort et al, 1980) and measles (Black, 1959), is that a small proportion of the population remains susceptible in the upper age classes. It is not known if social or genetic factors or artefacts arising from serological techniques are responsible for this observation (see Chapter 3, Part 1), but it raises doubt about assuming 100% exposure of the population to infection. The importance of resolving this problem stems from its effect on the estimated per capita rate at which susceptibles acquire infection in adult age classes; overestimated by case notifications if this observation from serology is true, or underestimated by serological data if the observation is an artefact of genetic non-susceptibility or of screening techniques. Accurate estimation of the force of infection in adult age classes (especially female) is most crucial for rubella,

because of its effect on the predicted impact of childhood mass vaccination upon the incidence of congenital rubella (an issue which is investigated in Chapter 6. See also Anderson & Grenfell, 1986).

In this discussion a number of doubts have been raised regarding the validity of using horizontal, age-stratified, case notifications in the study of rubella transmission dynamics. Age- and sex-dependent bias in reporting and the method by which age-prevalence profiles are represented confuse the interpretation of age-related trends in the force of infection and observations of the impact of selective vaccination on herd immunity to rubella. Quite obviously, age-prevalence profiles from notifications do not give a good picture of the degree of herd immunity to an infection in the community, particularly when affected by an immunization policy. These criticisms should be considered when comparing the authenticity of results derived from serological surveys and case notifications.

Chapter 5

ANALYSIS OF QUANTITATIVE MEASUREMENTS OF RUBELLA ANTIBODY

Part 1

RUBELLA ANTIBODY LEVELS IN S.E. ENGLAND AND S. YORKSHIRE

5.1 Introduction

The radial haemolysis (RH) test is a quantitative assay of serum antibody. The diameter of the zone of haemolysis is linearly related to the logarithm of specific-antibody concentration in a sample of serum (Russel, McCahon & Beare, 1975; Clarke et al, 1979). Simple modifications to the basic RH technique, which are detailed in the serological methods (Chapter 2), take advantage of this log-linear relationship and enable the rapid and reliable estimation of rubella antibody concentrations (standard iu/ml) in test sera.

As a result of these RH test adaptations, a substantial body of quantitative data was acquired during the screening of serum samples from the surveys of S.E. England (1980/84) and S.Yorkshire (1969-85) (see Table 3.1). In possession of these data it was, therefore, possible to make enquiries into the nature of changes, with age, in average rubella

antibody concentrations in the two study populations. The sort of questions addressed include the following:

(i) What is the rate of decay in mean antibody concentration for rubella in a community (if any)?
(ii) Do the average antibody levels and rates of decay differ between communities?
(iii) Is there variation in the rate of decay between individuals, and does this affect the heterogeneity in antibody concentrations on a population basis?
(iv) Are there differences between males and females?
(vi) How are community levels of antibody influenced by selective immunization?

These questions reflect an interest in resolving a number of problems which are of epidemiological concern. Firstly, there is a need to establish the reliability of age-dependent transmission rates which are based on age-serological profiles. Analysis of quantitative rubella data may provide information on the nature of seronegativity in the small proportion of individuals in the upper age classes (> 50 years) of age-serological profiles who lack detectable antibody (see Fig. 3.7; also Black, 1959; Wagenvoort et al, 1980; Anderson & May, 1985a). Secondly, investigations of the impact of vaccination on average antibody levels in a population, in relation to age-related trends in antibody levels, may indicate what threat exists of serious deterioration of vaccine-induced herd immunity. Thirdly, variation in decay rates between individuals and

between communities may provide leads on the importance of internal (genetic) versus external (environmental, social) factors in maintaining antibody levels in a population.

The following sections present the results of analyses of quantitative rubella data from the surveys of S.E. England and S. Yorkshire. Observations on age-related trends are then discussed in connection with the points of interest mentioned above, and also in relation to the implications to the design of future rubella vaccination programmes in this country.

5.2 Correlations to Age and Sex

Antibody concentrations (iu/ml) from rubella positive individuals, of all ages and both sexes, from the survey of S.E. England (estimated by the radial haemolysis test - Method I, see Chapter 2), crudely approximate to a log-normal frequency distribution (Fig. 5.1). Note that data for the two sources constituting this catchment area (see Table 3.1) have been pooled for the analysis, and for all subsequent analyses unless otherwise indicated. Comparable distributions were obtained for each of the yearly samples for S. Yorkshire (not displayed). The 'best-fit' normal distribution (see Davies, 1971, for computational details) was significantly different (goodness of fit chi-square test, Meddis, 1975) from the observed data for each survey ($p<0.001$ for S.E. England and $p<0.01$ for S. Yorkshire 1969 to 1985). Nevertheless, the overall pattern

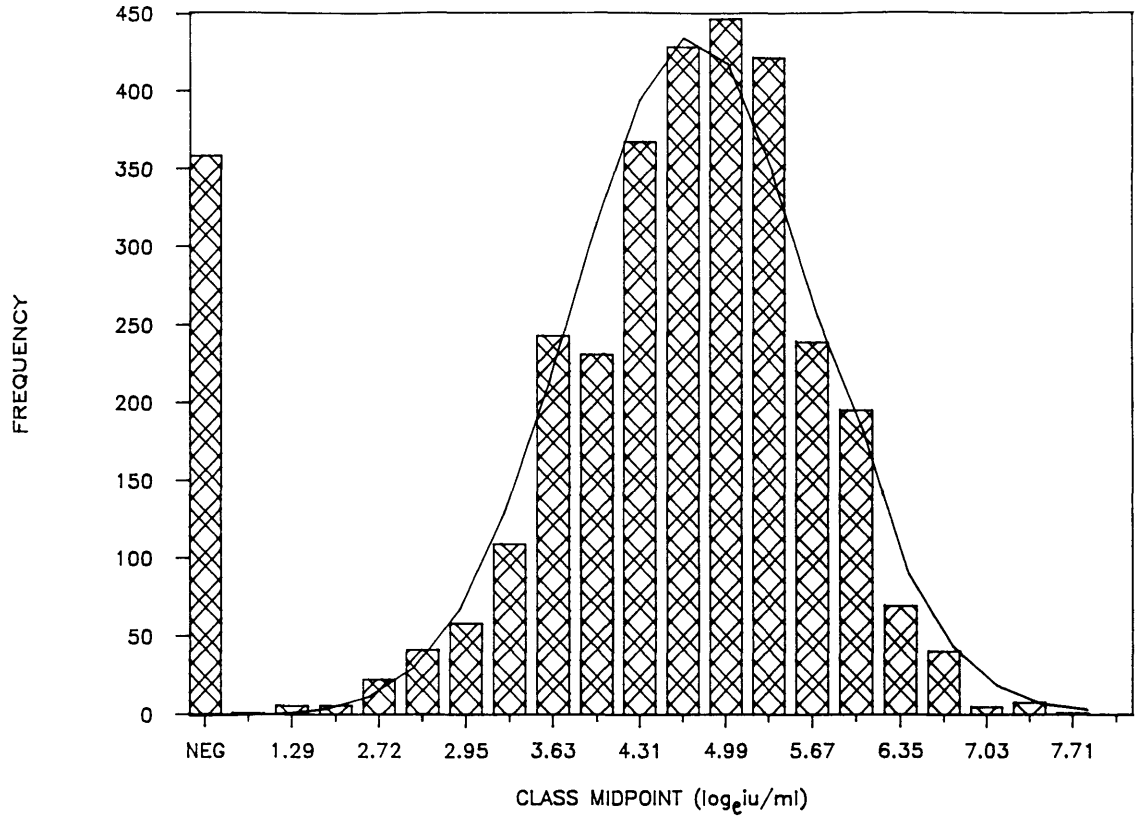


Fig.5.1 Observed frequency distribution of rubella (log-transformed) antibody concentrations (▨) and 'best-fit' normal distribution to these data (——) for samples from a survey of S.E. England 1980/84. Total frequency = 3310 individuals (males + females excluding those with maternal antibodies, i.e. <1 yr old). Threshold of sensitivity of serological technique is 3.3 iu/ml (see RH Method I, Chapter 2).

of these data approaches that of the normal distribution (goodness of fit statistic close to non-significance) and hence parametric statistical tests are used in this section.

Mean rubella-specific antibody concentrations were observed to decay with increasing age for males and for females from the S.E. England survey (Fig. 5.2). Regression analysis (see Table 5.1a) revealed a significant negative slope for both sexes ($p<0.001$). The variability in antibody levels within each age group (blocked up in samples of 50 individuals) is presented as the variance to mean ratio plotted against age, for males in Fig. 5.3a and for females in Fig. 5.3b. In each graph a trend for increasing heterogeneity in antibody levels, with age, is displayed.

Continuing with this line of enquiry, the probability of an individual possessing an antibody concentration less than 3.3 iu/ml (the arbitrary cutoff point for seropositivity using the RH test - Method I), may be estimated from the area beneath the expected normal distribution curve with upper limit of 1.2 ($\log_e$ 3.3 iu/ml) on the X-axis. When plotted against age, for each sex (Fig. 5.4a & b), there is an indication that the probability of falsely identifying, as seronegative, an individual who has experienced infection with rubella in the past, increases with age. Put another way, Fig. 5.4a & b suggest an increase in the possibility of some individuals losing immunity to rubella in the upper age classes (this, of course, depends on how well humoral antibody correlates with protection from re-infection).

Mean antibody concentrations for each of the years for

Table 5.1

Statistical analysis of quantitative data from S.E. England and for each year from S. Yorkshire.

(a) Regression analysis (only seropositive results from ages 1-95 years)

Survey	Year	Sex[1]	Slope (b)	Intercept (A)	Ratio[2] (F)	df (n-2)	p
S.E. England (Fig.5.2)	1980/84	M	-0.0141	5.303	75.58	1244	<0.001
		F	-0.0073	4.948	24.51	1677	<0.001
S.Yorkshire (Fig.5.5)	1985	T	-0.0177	5.582	89.77	391	<0.0001
	1981	T	-0.0160	5.321	50.76	362	<0.0001
	1979	T	-0.0155	5.440	52.84	361	<0.0001
	1976	T	-0.0185	5.314	64.26	320	<0.0001
	1973	T	-0.0145	4.899	38.56	357	<0.0001
	1969	T	-0.0157	5.249	51.04	425	<0.0001

(b) General statistics (seropositive results from ages 0-95 years)

Survey	Year	Sex	Mean [Ab][3] (log iu/ml)	Variance (S^2)	Mean Age (years)	# Samples
S.E. England	1980/84	M	4.754	0.814	38.9	1246
		F	4.694	0.836	34.8	1679
		T	4.720	0.830	36.6	2950
S. Yorkshire	1985	T	4.702	0.985	48.9	399
	1981	T	4.593	1.044	45.2	365
	1979	T	4.732	1.029	45.6	365
	1976	T	4.433	1.035	47.4	326
	1973	T	4.253	1.466	43.8	362
	1969	T	4.727	1.152	32.4	434

1. M = male, F = female, T = total samples;
2. Ho: b = 0; Ha: b < 0
   ANOVA F statistic (one tail, n-2 degrees of freedom (df))
   (Sokal & Rohlf, 1969);
3. Antibody concentration.

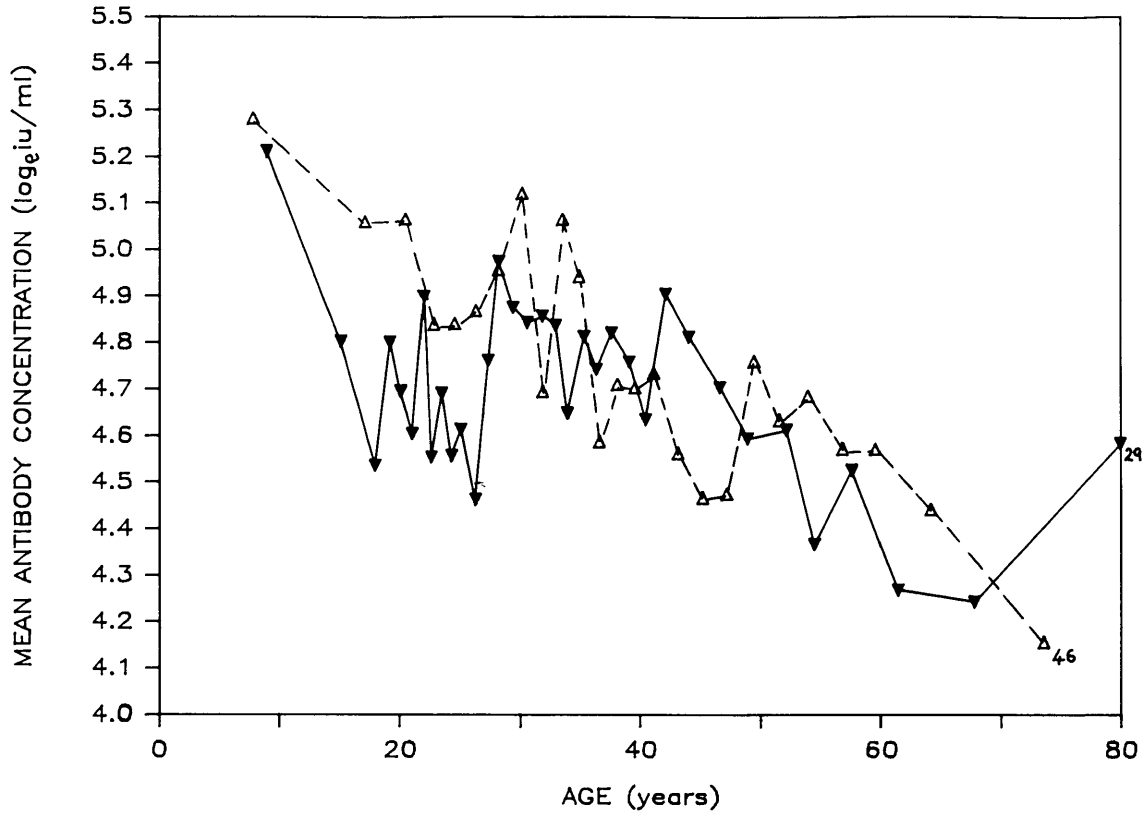


Fig.5.2 Decline in mean (log-transformed) rubella antibody concentration, with age, for males (Δ--Δ) and females (▼—▼) from S.E. England 1980/84. Each point denotes an average value for 50 seropositive samples (unless otherwise indicated).

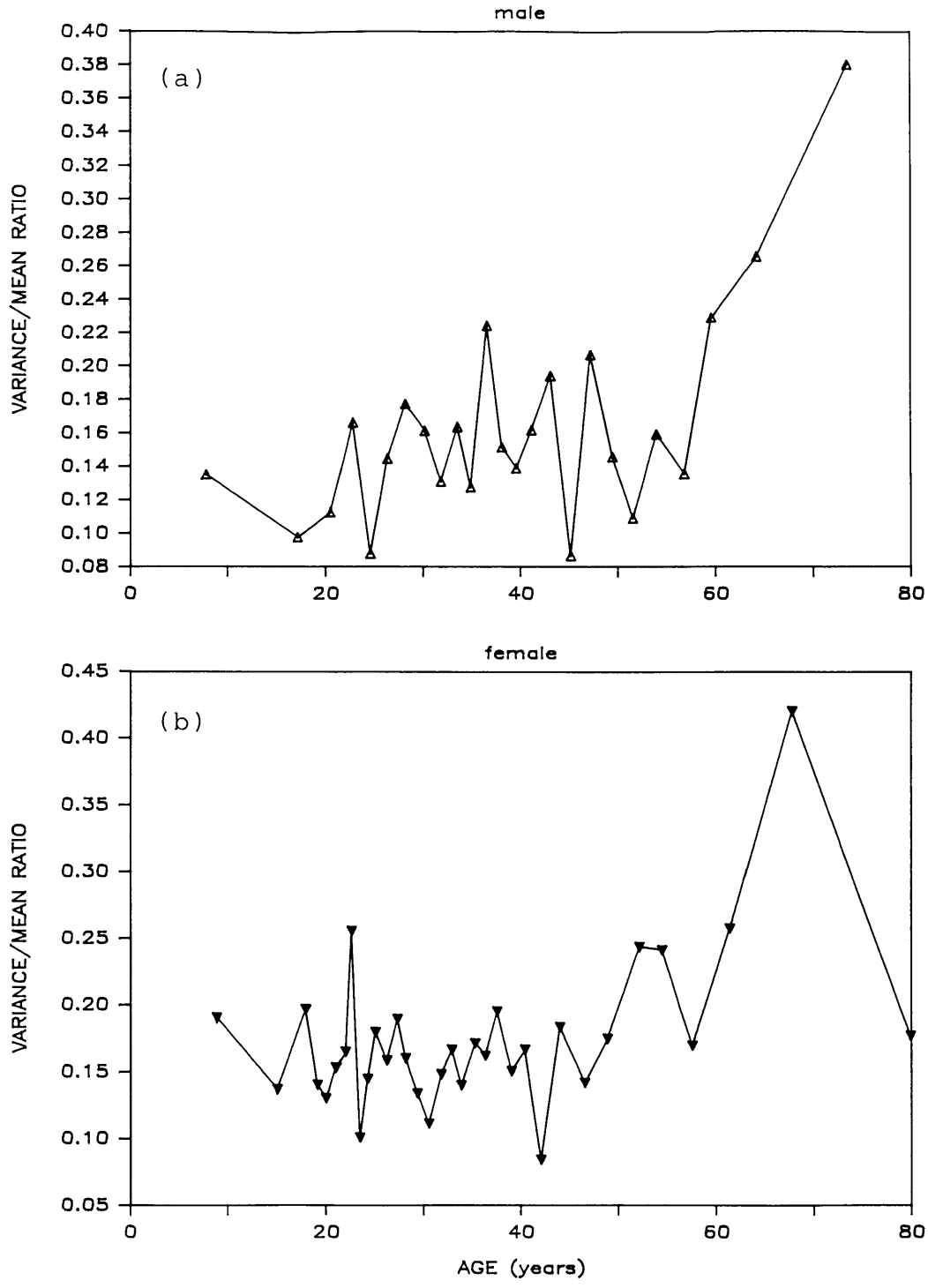


Fig.5.3 Trend in antibody concentration variability (denoted as the variance to mean antibody level ratio), with increasing age, for individuals in the S.E. England survey (1980/84). Graphs (a) and (b) record changes in the male and female segments of the study population, respectively (sample sizes are as for Fig. 5.2).

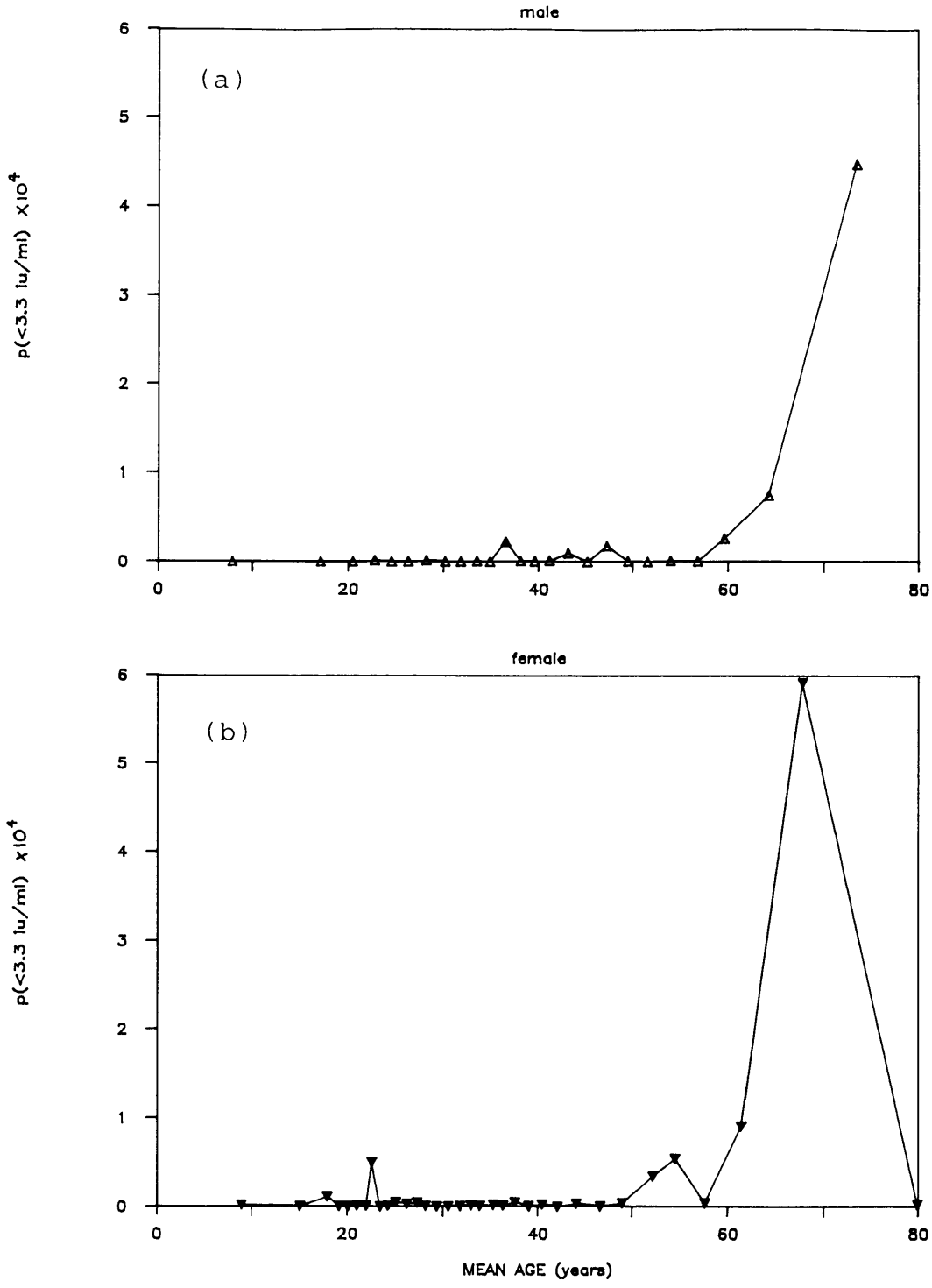


Fig.5.4 The probability, p, of falsely identifying, as seronegative, an individual with a past history of rubella infection whose antibody has decayed to an undetectable level. Changes in this probability are recorded, with age, for males (a) and females (b) from the survey of S.E. England (sample sizes are as for Fig. 5.2). Calculations assume antibody concentrations (log-transformed) to be normally distributed with a cutoff point for seropositivity of 3.3 iu/ml (RH Method I, Chapter 2).

which samples were obtained from South Yorkshire (1969, 1973, 1976, 1979, 1981 and 1985) exhibit a statistically significant decline, with age (Table 5.1a), as illustrated in Fig. 5.5. The rate of decay in antibody concentration for each year (total of samples - no segregation by sex), is similar (see Table 5.1a for regression coefficient values - calculated by linear regression), and they are comparable to that for males in particular from S.E. England, shown in Fig. 5.2 (the lower estimate of the regression coefficient for females, shown in Table 5.1a, is undoubtedly accounted for by the large proportion (> 1/3) of female samples with reduced average antibody levels over the age range 10-27 years, when compared with males of the same age - see Fig. 5.2). Consistent negative slopes for a number of surveys argues a common mechanism(s) whereby rubella antibody levels are maintained in individuals within a community.

In contrast, average antibody levels vary systematically from one year to another, and between the two survey populations. This is judged to be a manifestation of the duration of storage (at -20 C), and/or frequency of freezing and thawing, of the serum samples, rather than some biological or social difference between individuals of one survey year and another. Considerable robustness of rubella specific IgG, in serum samples, to repeated freezing and thawing was demonstrated by experiment and reported in the Serological Methods, Chapter 2. However, it should be pointed out that the time-span (1 year) and number of freeze/thaw operations (n=10) were not an adequate representation of the conditions to which some of the

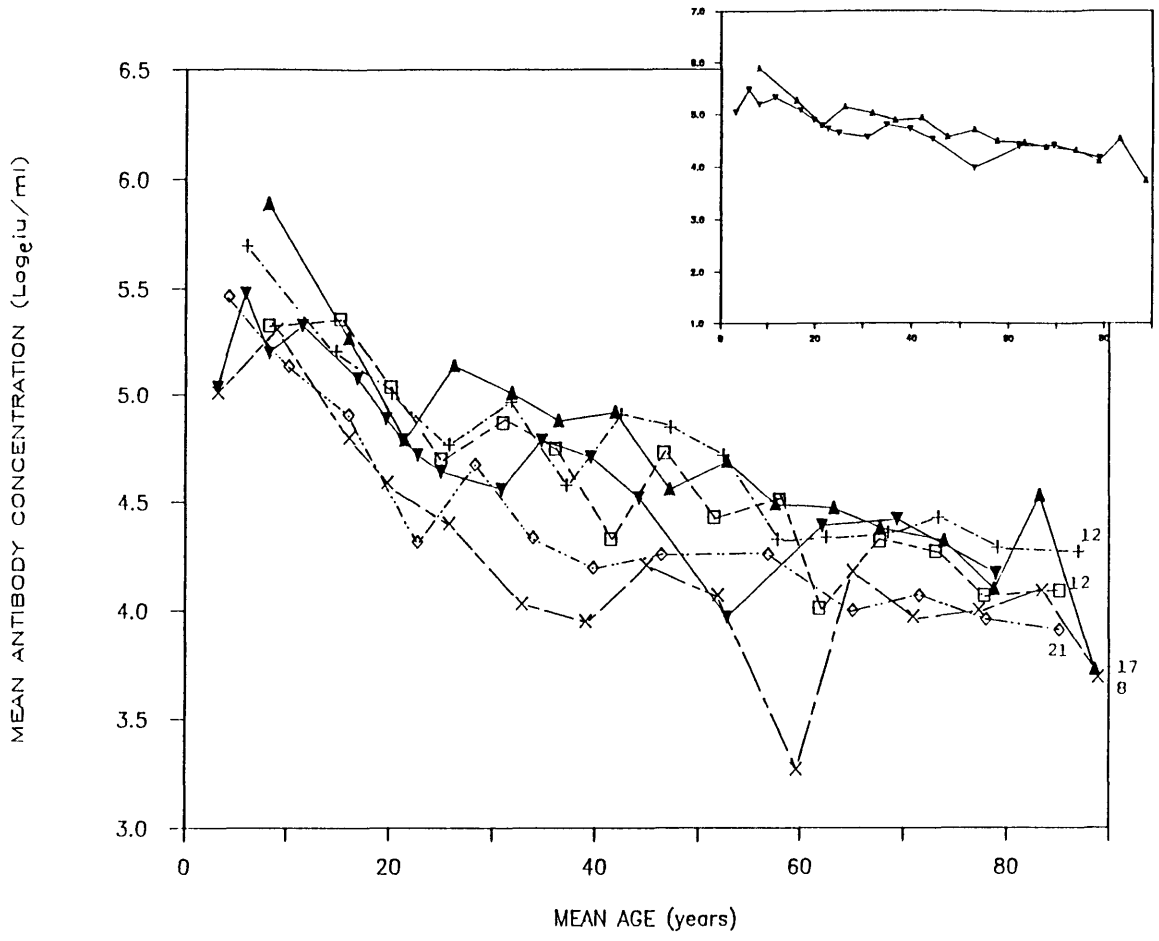


Fig.5.5 Decay, with age, in average rubella antibody concentration for individuals from S. Yorkshire, for a number of survey years: ▼—▼, 1969; ×--×, 1973; ◇--◇, 1976; +--+, 1979; □--□, 1981; ▲—▲, 1985. The results for 1985 and 1969, for which further analysis was later carried out (see text), are shown in the inset. Each observation point represents the average for 25 individuals (males and females pooled) unless otherwise indicated.

samples comprising these surveys had been subjected.

Generally, samples collected in more recent years were found to possess highest antibody levels (and least variation - see Table 5.1b). Consideration of the average age of individuals for each survey shown in Table 5.1b (necessary because of regression in antibody concentration with age) reinforces this argument. 1973 samples had particularly low antibody concentrations (Fig. 5.5 and Table 5.1b), and many more indeterminable serological results than for any other year, arising from deterioration of the specimens (see Table 2.3). For these reasons the data for this year were not presented in the form of an ASP in Part 1 of Chapter 3. Even between the two sources which constitute the S.E. England survey (Table 3.1) antibody concentrations differed significantly (two-tailed t test; $p<0.005$, excluding samples influenced by vaccination - see Fig. 5.2) over equivalent age ranges. Samples from the virology department, Kings College Hospital, were known to have been thawed and frozen on numerous occassions since collection in 1980, which may account for their lower mean antibody level compared with that for the serum samples from blood donors taken in 1984.

Further analysis on S. Yorkshire data is restricted to the two extreme years, representing pre- (1969 samples) and post- (1985) vaccination (see Fig. 5.5 inset). Consistent with the findings for S.E. England, variability in antibody concentration for both years (total data) shows an overall trend to increase with age (Fig. 5.6a & b); and the results presented in Fig. 5.7a & b suggest that there is a greater

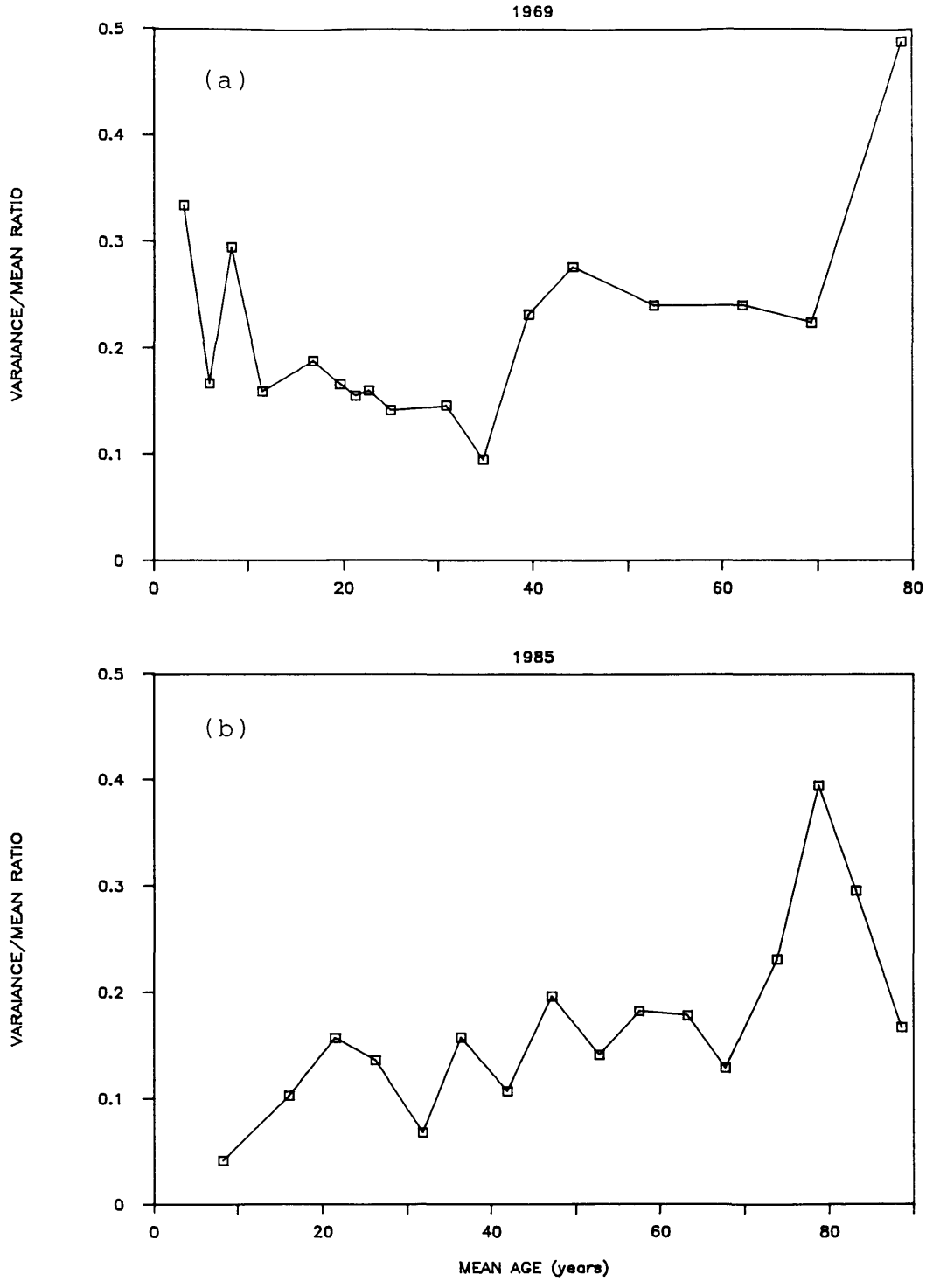


Fig.5.6 Changes in antibody concentration heterogeneity (shown as the variance/mean ratio), with age, in samples from S. Yorkshire 1969 (graph a) and 1985 (graph b). Sample sizes as in Fig. 5.5.

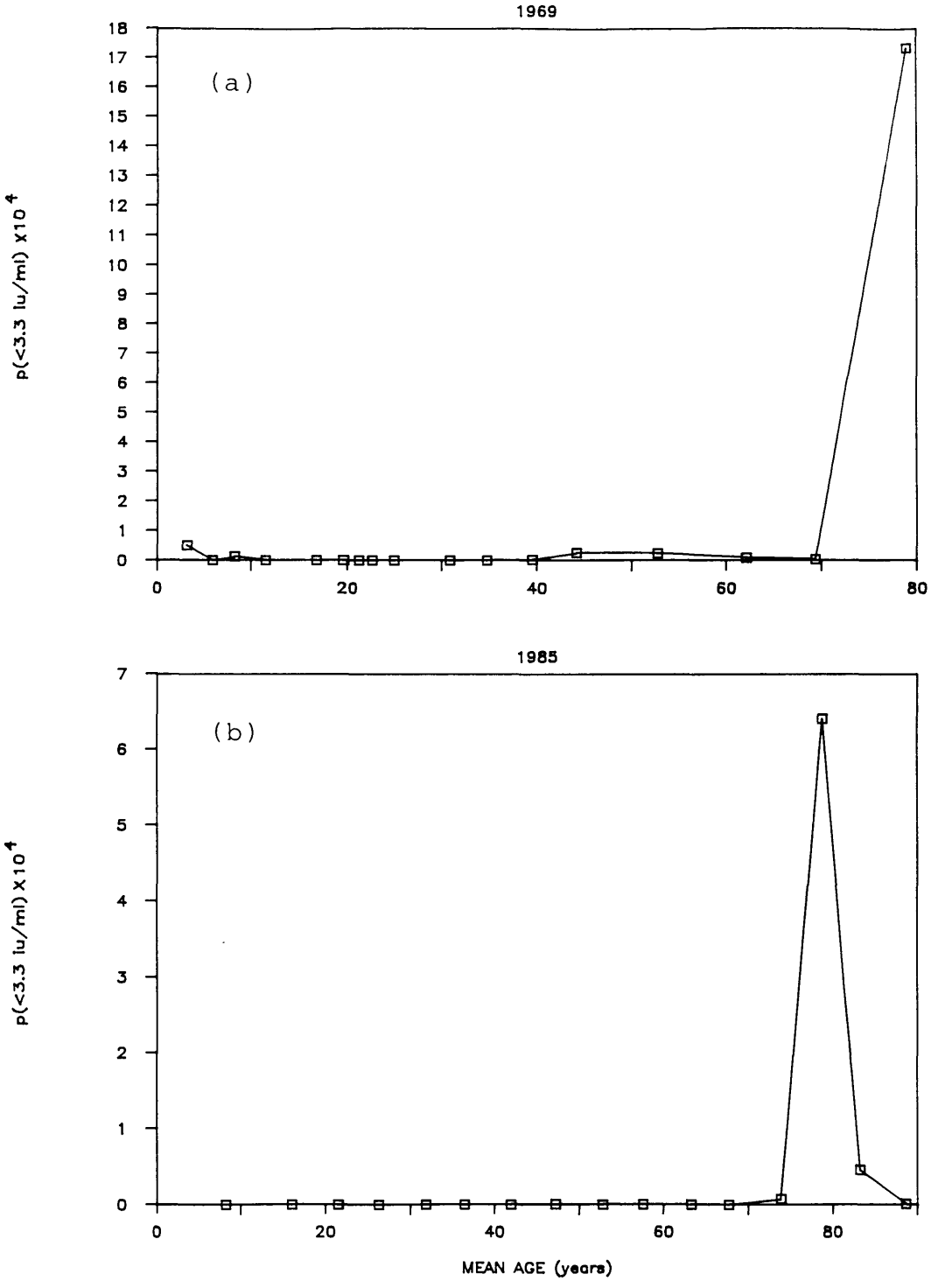


Fig.5.7 Changes, with age, in the probability of falsely identifying a rubella seropositive individual as seronegative (threshold of seropositivity set at 3.3 iu/ml) from S. Yorkshire 1969 (graph a) and 1985 (graph b). Sample sizes as in Fig. 5.5.

probability, in the older ages, of falsely identifying, as seronegative (i.e. < 3.3 iu/ml), an individual who has previously experienced infection. One point of interest, shown in Fig. 5.6a, is the degree of heterogeneity in antibody concentrations for young individuals (< 20 years) which exceeds that for the 1985 and S.E. England surveys.

5.3 Influence of Vaccination on Antibody Levels

The decline, with age, in mean antibody concentration in S.E. England, is broadly similar for male and female segments of the sample population (Fig. 5.2). However, for the range of ages corresponding to the years influenced by selective vaccination of 10-15 year old girls (10-27), the overall average (log-transformed) antibody concentration for females is significantly lower ($p<0.0001$, one-tail t test) than that for males of the same age. In contrast, no significant difference exists at the 5% level (two-tail t test) between the average antibody concentration for each sex for all ages other than 10-27 years.

Lower levels of specific antibody in rubella vaccinees when compared with those naturally immunized is a well known phenomenon (see, for example, Enders 1985; O´Shea et al, 1985; Spinner, 1984), and provides a plausible explanation for the differences between the sexes observed in this study. However, a proportion (exact percentage unknown) of the serum samples from Kings College Hospital were obtained from antenatal patients. Furthermore, the childbearing

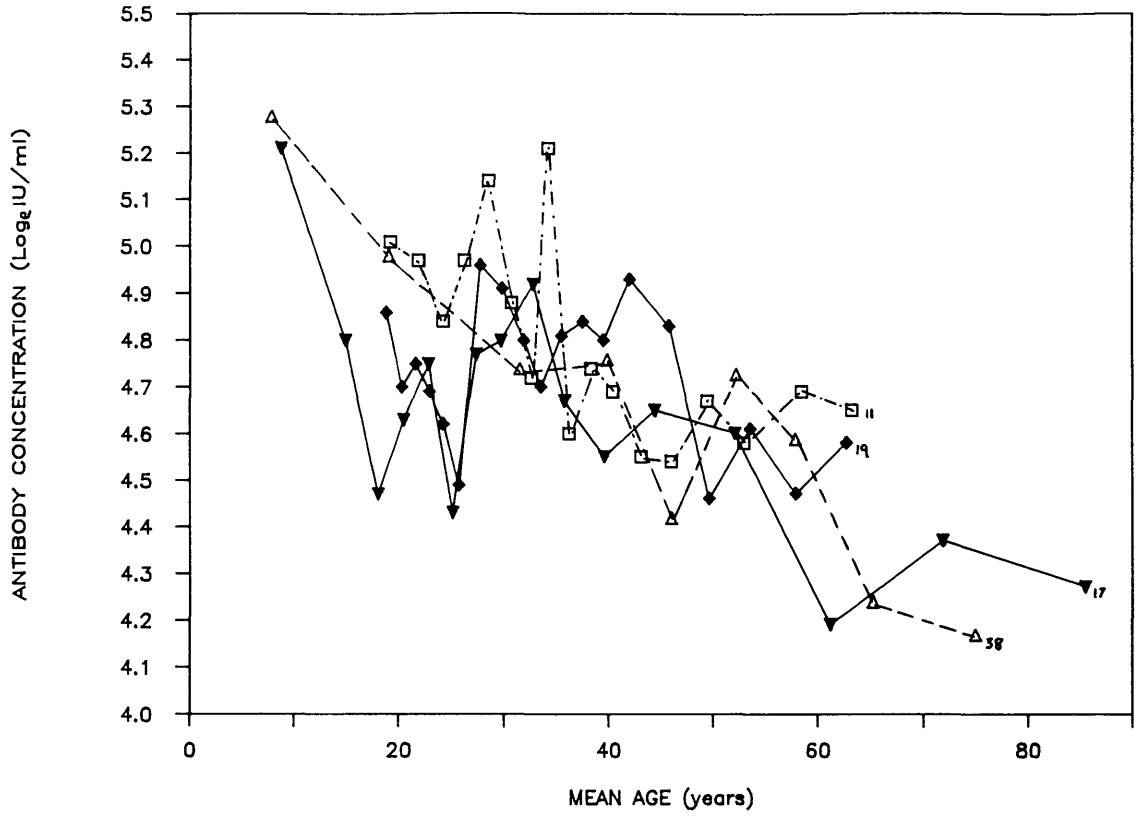


Fig.5.8 Low level rubella-specific antibody in vaccinees? - I. Decay, with age, in average antibody concentration for individuals from S.E. England stratified by sex and sample source: S. London BTC (1984) males, □---□, and females, ◆—◆; Kings College Hospital, London (1980) males, △---△, and females, ▼—▼. Each observation point represents a sample size of 50 individuals (except where indicated). Note the low level of antibody for females over the age range 10-27 years, compared with males of the same age, for both sources.

years (roughly 16-40) undoubtedly cover the upper end of the age range in question. Under these circumstances the possibility arises that low levels of antibody for females may be (in part) a consequence of haemodilution in pregnancy. Baboonian and Griffiths, 1983, demonstrated declining titres for a number of antiviral antibodies,including rubella, in pregnant women between first trimester and delivery. This they attributed to haemodilution.

In Fig. 5.8 the decline in mean antibody concentration, with age, is stratifed by sex, and by origin of samples for the S.E. England catchment area (i.e. Kings College Hospital and South London Blood Transfusion Centre, see Table 3.1). The reduced mean levels of antibody for females compared with males is seen over the same age range for both sources of sera. Blood is not donated by pregnant women (contraindicated by the National Blood Transfusion Service), and so these results endorse the hypothesis of low anti-rubella antibody levels in vaccinees.

Fig. 9a & b show the age-related decay in mean antibody level for males and females from the years 1969 (pre-vaccination) and 1985 (post-vaccination) for S. Yorkshire. The feature of importance is the relatively low levels of antibody in females, compared with males, over the age range corresponding to the main childbearing years (20-30). It seems highly probable that haemodilution in samples from pregnant women has had considerable bearing on these results, and indeed many of the female specimens from S. Yorkshire were received from women having antenatal

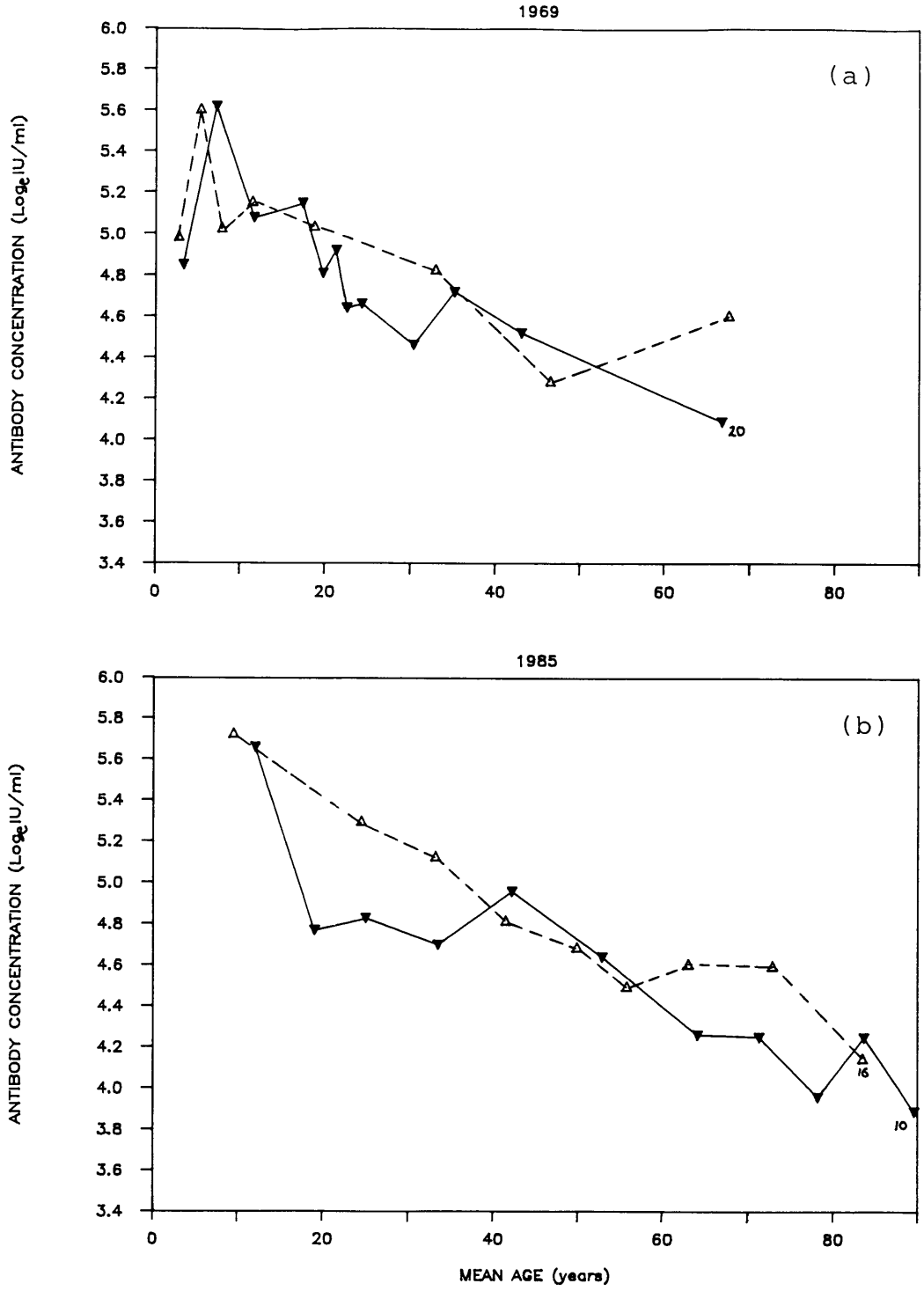


Fig.5.9 Low level rubella-specific antibody in vaccinees? - II. Decline, with age, in average antibody level for individuals from S. Yorkshire 1969 (grph a) and 1985 (graph b). Results for males are indicated by Δ--Δ and for females by ▼—▼ . Samples are blocked up into groups of 20 unless stated to the contrary.

syphilis screens (Dr.R. Jennings, Sheffield PHL, personal communication). However, the more exaggerated difference in antibody concentrations between the sexes (over the years affected by immunization) in the 1985 survey (Fig. 5.9b), compared with 1969 (Fig. 5.9a), would seem to be an indication of low level vaccine-induced antibody.

5.4 Discussion

The significant decline in mean rubella antibody concentrations, with age, and concomitant overall increase in heterogeneity of rubella-specific antibody levels, are crucial findings from the surveys of South Yorkshire and South East England. Persistence of anti-viral antibody following infection with childhood viruses, such as measles, mumps and rubella, is conventionally assumed to be lifelong. However, in view of these observed trends in antibody concentrations there arises the possibility that for a few seropositive individuals in the population, antibody concentration will decay, in time, to an undetectable level.

The age-serological profiles determined from the surveys of S.E. England and S. Yorkshire (see Fig. 3.7) show that a small proportion of the population remain seronegative for rubella in the upper ages (50+ years of age). This may be a consequence of the low force of infection in adult age classes (Fig. 3.8 & 3.10) possibly resulting from fewer interactions between older people and infectious (mostly

young) individuals.

However, estimates for the force of infection are, themselves, derived from the age-serological profiles. The results which have been presented here suggest that serological data may not always provide a true account of past exposure to infection, because in some individuals antibody will have decayed to an undetectable level. Consequently, the force of infection estimates for adult age classes may be underestimating the real transmission rates. Such an error could have considerable implications to the predicted outcome of a given mass vaccination policy (during which there is a tendency for the average age at infection to increase) on the number of cases of CRS in the population. Chapter 6, considers the effects of different force of infection estimates in adults during mass vaccination (see also Anderson & May, 1985a; Anderson & Grenfell, 1986).

Age-related decay in mean antibody concentrations have been documented for other childhood viruses, in countries where these infections are endemic. For instance, measles in the United States (Black, 1959) and mumps in the Netherlands (Wagenvoort et al, 1980). Both surveys measured neutralising antibodies which correlate well with IgG (Grist, 1979) as measured by the radial haemolysis test. These results suggest that the problems associated with the interpretation of serological data for rubella and the implications to the design of vaccination policy, may be relevant to other viruses for which there is an age-dependent risk of serious complications from infection

(e.g. mumps, see Anderson, Crombie & Grenfell, 1987).

The loss of detectable antiviral antibody should not automatically be assumed to correspond with a loss of immunity to re-infection, but may only reflect less than perfect sensitivity of the serological technique (see, for example, O'shea et al, 1981), or a test which is not measuring the major mediators of viral immunity. Evidence to substantiate this argument is not incontrovertible. However, relevant observations come from the resistance to primary rubella infection or re-infection by seronegative individuals following assumed exposure to the virus during epidemics (e.g. Brody et al, 1963) and following re-vaccination of supposed vaccine failures (Balfour et al, 1981). Resistence to mumps infection by seronegative adults exposed to the virus has also been reported (Wagenvoort et al, 1980). It is quite possible that cellular mechanisms of immunity, such as T cell cytotoxicity towards virally infected cells (Roitt, 1984), are more important in the defence against re-infection.

Specific antibody levels induced by vaccination are more often lower, and decay more rapidly, than those produced from rubella infection (O'Shea et al, 1985), although there is considerable variation between antibody class, vaccine type (RA27/3, $HPV_{77}DE_5$, Cendehill and To-336) and mode of vaccine administration (intranasally or subcutaneously) (O'Shea et al, 1985; Al-Nakib, Best & Banatvala, 1975). It, therefore, seems likely that in countries for which there is a rubella immunization programme, those segments of the population directly influenced by vaccination will have

lower average antibody concentrations than would be expected from natural immunization.

The weight of evidence from the surveys of S. Yorkshire and S.E. England suggests that this is indeed the case. The strongest indication comes from the samples from blood donors for whom the complication of haemodilution in serum from pregnant women was not present. Furthermore, quantitaive serological surveys of rubella antibodies in adult women in England (Spinner, 1984) and West Germany (Enders, 1985) provide a similar picture. For example, in both of these studies, marked increases in the number of females with low antibody titres (<32) could be attributed to vaccination.

The important epidemiological (and medical) consideration is how well these lower levels of antibody protect against re-infection. As was mentioned in section 2.4 it is generally supposed that any level of specific antibody indicates the presence of memory cells which will be stimulated on re-exposure to virus and prevent viraemia. In accord with this the cutoff point of immunity (or susceptibility) for this study was set at the threshold of sensitivity of the radial haemolysis test. Studies show that low levels of naturally induced rubella IgG antibody do, almost invariably, provide protection from challenge infection (Mortimer et al, 1981; O'Shea, Best & Banatvala, 1983). However, there is no conclusive evidence of solid protection from low level, or any level, of vaccine induced antibody to rubella (Harcourt, Best & Banatvala, 1980; O'Shea, Best & Banatvala, 1983). The challenge study of

O´shea, Best & Banatvala (1983) reported either viraemia or excretion of virus into nasopharyngeal secretions (which are the prerequisites for transplacental and horizontal transmission, respectively) for a number of vaccinees with low level antibody (<15iu/ml).

Unless a very high rate of vaccination of infant boys and girls is a realistic objective (as part of a dual stage rubella immunization policy), then the doubts raised over the degree of protection afforded by vaccine induced antibody, and declining antibody concentrations with age, argue for continuation of the current UK selective programme of immunization (10-15 year old girls). Under this policy herd immunity is largely the product of natural infection (with solid immunity), and immunity from vaccination need only last 2-3 decades (compared with 3-4 decades were mass vaccination to be implemented) to prevent vertical transmission and, therefore, CRS.

The rate of decline in mean antibody concentration for the S.E. England and S. Yorkshire surveys were found to be comparable, suggesting a similarity in the factors involved in maintaining antibody levels. Heterogeneity in specific antibody titres following rubella infection (Champsaur, Dussaix & Tournier, 1980; Morgan-Capner, Burgess & Fisher-, Hoch, 1982) indicate a genetic involvement in the primary immune response. Inherited characteristics may also determine the rate of decay of antibody since the variability in antibody concentrations increases with age (Fig. 5.3 and 5.6). The possibility of genetic association with the rubella humoral response is considered in detail in

the second part of this chapter.

Environmental factors may play a part in maintaining rubella antibody concentrations by the boosting of antibody titres upon re-exposure to infection. It is possible that declining antibody levels, with age, are a consequence of concurrent reduction in the frequency of contacts between immune and infectious individuals and, therefore, fewer boosts to antibody production.

Following an epidemic, or after experimental challenge, stimulation of rubella antibody production has been observed - although more commonly in vaccinees than in people possessing naturally acquired antibody (Harcourt, Best & Banatvala, 1980; Horstmann et al, 1970; O´Shea, Best & Banatvala, 1983). Evidence from island communities invariably shows that natural immunity to re-infection persists for many years in the absense of re-exposure from viruses such as measles, mumps and rubella. For example, in the study of a rubella epidemic on St. Paul island, Alaska, in 1963 (Brody et al, 1965), immunity was virtually solid in individuals who were last exposed to the virus in an epidemic 20 years previous. During measles vaccine trials on the same island , Brody et al (1964) reported no cases of re-infection in seropositive subjects who had not been exposed to measles virus for at least 21 years. Over 60 years without mumps exposure did not result in a loss of immunity to this virus in old people on St. George island, Alaska (Reed, 1967).

Furthermore, most antibody titres of island inhabitants do not seem to decay any more rapidly than would be the case

in endemic areas (e.g. Black and Rosen, 1961) and do not show significant rises in titre upon re-exposure or vaccine challenge (Brody et al, 1964; Levitt, 1970).

The contribution made by re-exposure to natural infection in maintaining antibody concentrations in a population is of relevance to the design of vaccination policy. Mass vaccination at a young age will reduce the circulation of wild virus and , if the goal of eradication is achieved, eliminate the influence of re-exposure in maintaining antibody levels. Were this influence crucial to the persistence of immunity (although, from the evidence given above, this seems unlikely for rubella) then the population would become vulnerable to re-introduction of the virus. Such problems would not arise under a selective vaccination policy.

Part 2

GENETIC ASSOCIATION WITH RUBELLA-SPECIFIC ANTIBODY

5.5 Introduction

In the first part of this chapter the analysis of age-related changes in rubella antibody concentrations, from a number of serological surveys in England, consistently recorded a trend for increase in antibody-level heterogeneity with an increase in age. This observation suggests that the rate of decay in antibody concentration, with age, differs from one individual to the next. Genetic variation between individuals provides one possible explanation for these results.

Evidence from various other sources implicates genetically determined factors in the humoral response to viral infections. For example, the considerable heterogeneity in antibody response between individuals following primary infection with viruses, such as rubella (Champsaur, Dussaix and Tournier, 1980; Morgan-Capner, Burgess & Fisher-Hoch, 1982), is almost certainly (in part) of genetic origin. Associations between the presence or absence of specific histocompatibility markers and the antibody response in individuals following vaccination against influenza A (Spencer et al, 1976) or rubella

(Ishii & Nakazono, 1984; Kato et al, 1982; Spencer et al, 1977) have been documented. Furthermore, from a study of anti-viral titres in the Orkneys, Poskanzer et al (1980) reported that HLA alleles explained a significant proportion of the variability seen in antibody titres for measles and rubella viruses in a small number (50) of normal, naturally immune individuals.

The above observations provided the stimulus and supportive evidence for a further, more extensive investigation of a genetic association with the rubella antibody response. The incentive to firmly establish such an association, from an epidemiological standpoint, comes from the need to resolve the various factors that may generate patterns of age-dependency in rubella transmission rates (see Chapter 3).

Such patterns are thought to arise from heterogeneity in contact rates in a community (Fine & Clarkson, 1982b; Anderson & May, 1983; Schenzle, 1984; Grenfell & Anderson, 1985). However, Anderson & May (1985a) argue that the shape of age-serological profiles used to determine age-related forces of infection, may result from genetic non-susceptibility of some individuals, or from genetically determined variation between individuals in the rate of decay, with age, in antibody concentration (the latter scenario is suggested by the results presented in Part 1 of this chapter).

In the study presented here, rubella antibody levels in large numbers of serum samples from individuals either naturally immune (or susceptible) to rubella virus infection

(the vast majority) or vaccinated against rubella are analysed with respect to HLA and blood group antigens.

Two approaches have been taken in the analyses, dependent upon the characteristics of the tissue typed subjects from which serum specimens were obtained. Phenotype frequency analysis, according to rubella antibody concentration, was undertaken for samples from unrelated individuals who were of two distinct types - young and adult. For family group data, however, an assessment was made of the deviation from random segregation of HLA haplotypes (combinations of alleles an chromosome 6) in siblings with similar rubella antibody levels.

These analyses will provide information on the importance of inherited characteristics as determinants of the rate of decay, with age, of rubella antibody in the individual and the level of herd immunity in a defined population. The relative contributions of genetic as opposed to social or environmental factors (discussed in the section 5.4) may then be assessed.

5.6 Subjects and Methods

(i) Subjects and sources

Samples of serum (or in one case citrated plasma) have been obtained from individuals for whom HLA tissue typing, and, in some cases, blood group (ABO and Rhesus) testing, had previously been carried out. These individuals may be

divided into two distinct groups; those that are unrelated and those associated into family units. Sources for each of these groups, together with some important characteristics, are detailed in Tables 5.2 and 5.3.

Bone marrow transplant volunteers from the two blood transfusion centres (SEBTC and SWBTC, see Table 5.2) form a subset of unrelated people representing a random selection of healthy adults (generally speaking of blood doning age) from the population. These attributes distinguish them from the juvenile chronic arthritis (JCA) patients, 65% of whom were less than 15 years of age.

Citrated plasma samples for 177 members of 30 families forming part of a study investigating genetic associations to schizophrenia (Table 5.3), were donated to this study. Serum samples from a second, much larger, family study of rheumatoid arthritis, comprising 78 families and a total of 575 individuals, who had been comprehensively tissue typed, were also made available (Table 5.3).

(ii) Experimental methods

All samples had been kept at -20^{o}C since time of bleeding. Each specimen was screened to determine the rubella-specific antibody concentration (iu/ml) using the radial haemolysis Method II described in Chapter 2. Two estimates of experimental reproducibilty were determined, in addition to test-to-test variability monitored throughout the practical procedures:

Table 5.2

Survey description: tissue-typed samples from unrelated individuals

Sample source (abbreviation)	Description of samples (number)	Year bled	Details on: age (yr)	sex	origin	Reference on typing methods
Dr.B. Bradley UK Transplant Service South West Regional Transfusion Centre Bristol (SWBTC)	Healthy adult volunteer bone marrow donors (368)	1984-6	19-43	YES	UK	Bender, 1984
Mr.K. Sherwood South London Blood Transfusion Centre (SEBTC)	Healthy adult volunteer bone marrow donors (477)	1984-6	19-48	YES	UK	Bender, 1984
Mr.S. Burman Division of Rheumatology MRC Clinical Research Centre, Harrow (CRC)	Juvenile chronic arthritis patients (152)	1981-4	2-28	YES	UK	Hall et al, 1986

Table 5.3

Survey description: tissue-typed samples from family groups

Sample source (abbreviation)	Description of samples (#fam #ind)	Year bled	Origin	HLA loci typed	Reference
Dr.H. Chapel Dept. Immunology John Radcliffe Hospital Oxford (JRH)	Schizophrenic families (42 177)	1977	UK	A B	Williamson, 1979
Dr.W. Ollier Dept. Immunology London Hospital Whitechapel, London (LHW)	Rheumatoid arthritis families (73 575)	1984-86	UK	A B C DR MT DQ D	

(a) Duplicate serum samples, separated by a period of approximately six months, were taken from 150 of the volunteers from SEBTC. The second sample of each pair was screened for rubella antibody without prior knowledge of the first. The resulting two sets of data were compared using a 'related' t-test (Meddis, 1975) to verify the hypothesis that the mean difference between each pair is not significantly different from zero.

(b) The 177 samples from the Schizophrenic family study (see Table 5.3) were screened in two separate test-series (i.e. on separate occasions), from which was determined Spearmans' coefficient of rank correlation (R^2).

Citrated plasma and serum specimens were taken from 5 volunteers (these individuals provided the control sera described in Chapter 2). Rubella antibody concentrations were evaluated in a single test to determine any difference between the two types of sample.

Standard tissue typing and blood grouping techniques were used by the various research departments who supplied the specimens; these have been detailed in publications referred to in Tables 5.2 and 5.3.

(iii) Analytical methods

Phenotype frequencies of particular HLA alleles, or blood groups, for samples from unrelated individuals grouped according to specific rubella antibody levels or, according

to whether, or not, influenced by vaccination, are compared using Fisher's 2×2 Exact test, or a 2×k chi-square test (Meddis, 1975).

For family data, the method of Green and Woodrow (1977) is used to test the significance of departure from a null hypothesis of random segregation of HLA haplotypes (sets of HLA or associated alleles, on chromosome 6, that are inherited en masse) in siblings categorised according to rubella antibody concentration. Briefly the test procedure is as follows. Choosing only subjects from one rubella antibody category (e.g. high antibody potency), scores are given to each sibship of two or more individuals corresponding to the number of inherited haplotypes they share. The total score, S, for all families in the study group is then compared with the mean score, μ, (with variance, σ^2), expected under the above stated hypothesis, by calculating the statistic T, where

$$T = (S - 0.5 - \mu) / \sigma$$

The significance of the result is assessed by consulting normal integral tables (T is approximately distributed as a standardized normal variate). The null hypothesis of random zygotic assortment of HLA haplotypes (i.e. assuming simple Mendelian inheritance) in these families is rejected if T is greater than or equal to the value given in tables at the level set for statistical significance (this implies a one tailed test). Under such circumstances it is inferred that there is some disturbance of haplotype segregation in those

siblings falling into a specific category of rubella antibody concentration. Criteria for categorising individuals, from unrelated and related sample sources, are listed in Table 5.4.

5.7 Antibody Determination and Reproducibility

Very few problematic RH readings were observed from the initial screening of samples (Table 2.3), which is indicative of their good condition. Equivocal results were re-tested by RH and if still unresolved were deleted from the statisical analysis. Citrated plasma samples produced zones of haemolysis, on average, 0.5 mm less than serum contols, and, antibody concentrations of the samples from the schizophrenic study were adjusted accordingly. No qualitative differences were observed between specimens of serum and plasma.

The mean difference in rubella antibody concentrations of 150 paired serum samples from SEBTC was found not to differ significantly from zero at the 5% level when analysed by a ´related t test´ (two tailed). The relative positions of the 177 schizophrenic samples, ranked according to rubella antibody concentration, were found to be highly correlated (Spearman´s Coefficient, $R^2 = 96.3\%$) to their rank order after a second, separate, rubella screen. Inter-test variability for the RH test Method II is recorded Table 2.1b.

Table 5.4

Criteria by which samples from each source (described in Tables 5.2 & 5.3) are divided into groups for analysis

Criterion	Characteristics of group	Abbreviation
1	[Ab][1] > Median	HIGH
2	2.5[2] iu/ml ⩽ [Ab] < Median	LOW
3	[Ab] ⩾ Upper Quartile	VHIGH
4	2.5 iu/ml ⩽ [Ab] ⩽ Lower Quartile	VLOW
5	[Ab] ⩾ 2.5 iu/ml	POS
6	[Ab] < 2.5 iu/ml	NEG
7	Vaccination influence (only females aged 10-30 years)	VAC
8	Without vaccination influence (no females aged 10-30 years)	~~VAC~~

1. [Ab], antibody concentration;
2. 2.5 iu/ml, cutoff point for seropostivity using the radial haemolysis test - Method II (Chapter 2).

5.8 Association Analysis Results

(i) Unrelated samples

Phenotype frequencies for HLA-A, B, C & DR locus alleles are recorded for each group of unrelated individuals, by their respective sources, in Table 5.5. The number of loci and alleles typed differs between the three sources. However, the 'broad' specificities for locus A and B are, in general, represented for each group. Antigen splits, known as 'small' specificities have been identified only where better defined antisera were available, and these are shown indented in Tables 5.5a & b. For comparison, figures in the last column of Tables 5.5a-d represent control phenotype frquencies of each antigen specificity determined for large samples of European caucasians (EUC). These data were taken from the report of the 1980 Histocompatability Workshop (Terasaki (ed.), 1980).

HLA antigen frequencies for bone marrow donors were found to be broadly similar for the two sources in England (SEBTC and SWBTC), and comparable to the EUC population estimates for HLA-A, B and C loci (Tables 5.5a-c). A few markedly discrepant antigen frequencies, compared with controls, are observable, e.g. Cw7. However, in view of the small number of volunteers sampled (relative to the EUC studies) and the large number of specificities tissue typed, it remains a reasonable assumption that these sample groups of bone marrow donors are representative surveys of normal unrelated adult caucasians in this country.

Table 5.5

HLA-A, B, C and DR phenotype frequencies[1.](%) in samples of individuals, all of UK origin, from three sources in England and from a control study of European caucasians (EUC) (Terasaki (ed.), 1980).

(a) Locus A

HLA Antigen broad/small[2.]	Sample source[3.] SEBTC n=476	SWBTC n=368	CRC n=152	Control EUC n=2648
A1	38.2	32.6	32.2	27.5
A2	44.4	47.3	68.4	45.3
A3	29.8	29.1	21.1	21.9
A9	17.8	22.3	19.1	
Aw23[4.]		11.4	2.0	4.5
Aw24		7.9	16.5	18.2
A10	8.4	8.4	6.6	
Aw25		2.2	1.3	3.7
Aw26		5.4	5.3	7.2
A11	6.9	10.3	9.9	11.5
A28	6.5	6.3	3.9	7.7
A19	21.4	25.0	21.7	
A29	8.4	10.6	4.6	7.4
Aw30		2.4	4.6	4.7
Aw31		0.5	5.9	5.4
Aw32	7.3	5.7	4.6	8.8
Aw33		0.8	1.3	3.3
# Antigens tested for	9	14	14	

1. Phenotype frequency $= \frac{\text{\# individuals with HLA antigen}}{\text{\# individuals tested}} \times 100$
2. Small antigen specificities are 'splits' of the broad antigens (resultant from improved test sensitivity);
3. Abbreviations as in Table 5.1;
4. w, as in HLA-Aw32, Bw21..., indicates a provisional specificity.

(b) Locus B

HLA Antigen	Sample source			Control
broad/small	SEBTC n=476	SWBTC n=368	CRC n=152	EUC n=2652
B5	10.1	9.5	10.5	
B51			9.9	13.9
Bw52			0.7	2.9
B7	24.3	27.2	13.8	16.8
B8	28.9	27.5	28.3	15.7
B12	27.3	37.0	27.0	
B44		36.4	25.7	20.7
B45		0.3	0.7	2.2
B13	4.6	1.9	3.9	5.6
B14	7.7	7.1	2.0	5.8
B15	14.3	12.8	15.1	
Bw62		10.3	13.8	10.4
Bw63		0.3	1.3	1.0
Bw16	2.9	3.3	16.5	
B38			2.6	5.0
B39			13.8	4.1
B17	11.9	9.5	2.0	
Bw57			2.0	6.2
Bw58			0.0	2.2
B18	6.9	4.9	6.6	11.2
Bw21	3.6	3.0	3.3	
B49			2.0	4.5
Bw50			1.3	2.5
Bw22	7.1	3.0	3.9	
Bw55			2.6	4.4
Bw56			1.3	1.1
B27	8.2	7.1	11.2	7.7
B35	9.0	13.9	15.1	18.2
B37	2.9	1.9	2.6	3.0
B40	10.3	14.1	27.0	
Bw60			23.7	6.7
Bw61			2.6	3.3
Bw41			2.0	2.0
B47	0.8	0.3		0.9
# Antigens tested for	17	19	25	

(c) Locus C

HLA Antigen	Sample source		Control
	SEBTC n=319	CRC n=152	EUC n=2651
Cw1	4.7	10.5	8.1
Cw2	7.8	9.2	10.0
Cw3	28.2	36.2	19.1
Cw4	14.7	15.1	22.7
Cw5	16.9	22.4	11.7
Cw6	19.1	9.9	15.1
Cw7	28.2	32.9	4.5
Cw8		1.3	3.8
# Antigens tested for	7	8	

(d) Locus DR

HLA Antigen	Source	Control
	CRC n=152	EUC n=2499
DR1	19.1	13.3
DR2	13.8	25.1
DR3	30.3	20.4
DR4	15.8	18.3
DR5	25.0	19.5
DRw6	29.6	4.3
DR7	12.5	23.4
DRw8	36.2	5.4
DRw9	5.3	2.2
# Antigens typed for	9	

For JCA (source CRC) patients the phenotype frequency of a number of specificities, for example, HLA-A2, Bw60, DRw6 & DRw8, (see Table 5.5), was markedly different from that of the EUC control or from those of adult voluteers from SEBTC or SWBTC, particularly in the DR locus. Comparable results have been published by Hall et al (1986), in a study of the genetic susceptibility to early onset pauciarticular juvenile chronic arthritis, from which the majority of the 152 samples used in this present analysis were derived.

Considerable difference is observed in the mean rubella antibody concentration for CRC samples (see Table 5.6), compared with either set of blood transfusion centre samples (one tailed t test; differences are significant at $p<0.0001$), and the same situation exists for their respective variances (F ratio test; significant inhomogeneity at $p<0.01$). This may be attributed to to the young age of JCA patients (mean age of 12.9 years); the volunteer donors are all adults (mean age of 28 years). The high degree of heterogeneity in antibody concentrations in samples from young individuals from CRC (Table 5.6) finds some corroboration from the survey of S. Yorkshire 1969 analysed in the Part 1 of this Chapter (see Fig. 5.6a, note the relatively large number of samples for the younger age groups).

Possible associations between HLA alleles and rubella specific antibody concentration (with or without the influence of vaccination) are recorded in Table 5.7. These results are obtained by comparing phenotype frequencies (for all HLA alleles in each locus tested) in subsets of samples

Table 5.6

General statistics: HLA typed unrelated samples
(calculations are based on seropositive results only)

Source	Rubella Ab Status #Pos	#Neg	Rubella $[\overline{Ab}]^{1.}$ (log_eiu/ml)	Mean Age (yrs)	Variance S^2	Ratio $S^2/[\overline{Ab}]$	Median [Ab] (iu/ml)
SEBTC	456	21	5.193	28.0	0.749	0.144	201.3
SWBTC	349	19	5.016	30.0	0.657	0.131	156.3
CRC	100	52	5.648	11.5	1.059	0.188	292.1

1. Mean antibody concentration

(grouped according to criteria set out in Table 5.4) from each source in Table 5.2. Comparisons of frequencies of these genetic markers are evaluated by Fisher's 2×2 Exact test. Only significant ($p<0.05$) associations are reported. It is important to mention that every type of comparison considered is represented in Table 5.7, and samples from each source (CRC, SEBTC and SWBTC) have been analysed separately and identically.

Table 5.7 lists a number of associations between locus A or B antigens and rubella antibody levels in normal, unrelated adult samples from the two blood transfusion centres. HLA-A1 is associated with high rubella antibody concentration (> median) in subjects from SEBTC. The same is true for HLA-A3 but only in females, from the same source, who fall within the age range most influenced by vaccination (10-30 years). In contrast, the frequency of HLA-B5 is high in SWBTC subjects possessing (a) very low, positve, antibody levels ($\leqslant$lower quartile) or (b) low positive antibody concentrations (<median) but excluding results from individuals influenced by vaccination (i.e. females aged 10-30 years). HLA-B27 is statistically more frequent in individuals seronegative for rubella, compared with those seropositive.

These are, however, only weak associations, whose significance is lost when corrected for the number of antigen specificies typed (see p_c values in Table 5.7). Confidence in these associations is further reduced because the results for the two study groups do not match each other, even though the subjects for each source were drawn

Table 5.7

Possible associations between HLA alleles and rubella-specific antibody concentration. Phenotype frequencies in individuals from three study samples (see Table 5.2), grouped according to the criteria set out in Table 5.4, are compared using Fisher's 2x2 Exact test. Only statistically significant results ($p < 0.05$) are recorded.

HLA antigen	Comparison Group A pf[1]	#	vs Group B pf	#	Sample source	Significance of association z[2]	p[3]	p_c[4]
A1	HIGH		LOW		SEBTC	2.45	1.43×10^{-2}	NS[5]
	44.4	225	32.7	233				
A3	HIGH/VAC		LOW/VAC		SEBTC	2.60	9.32×10^{-3}	NS
	39.4	66	21.5	65				
B5	VHIGH		VLOW		SWBTC	2.07	3.85×10^{-2}	NS
	3.2	95	10.9	92				
	HIGH/~~VAC~~		LOW/~~VAC~~		SWBTC	2.21	2.71×10^{-2}	NS
	4.8	126	12.6	127				
B27	POS		NEG		SWBTC	1.98	4.77×10^{-2}	NS
	6.3	349	21.1	19				
DR4	POS		NEG		CRC	3.32	9.1×10^{-4}	5.0×10^{-2}
	8.1	99	30.2	53				
DR5	HIGH		LOW		CRC	3.48	5.04×10^{-4}	2.72×10^{-2}
	48.9	45	12.2	49				
	VHIGH		VLOW		CRC	2.70	7.0×10^{-3}	NS
	40.0	25	4.0	25				
	HIGH/VAC		LOW/VAC		CRC	2.52	1.18×10^{-2}	NS
	40.7	27	7.4	27				

1. Phenotype frequency, pf; number of individuals in group possessing the specific HLA allele, #.
2. Normally distributed z statistic of Fisher's 2x2 Exact test.
3. Probability, p, of score occurring by chance (2 tail).
4. Corrected probability, p_c, ($p \times$ number of antigens typed for - see Table 5.5).
5. Not significant, NS.

from very similar populations (compare phenotype frequency and statistical data in Tables 5.5 and 5.6, respectively).

For patients with JCA two distinct types of association between alleles of the HLA-DR locus and levels of rubella antibody were identified, as shown in Table 5.7. The frequency of HLA-DR4 is high in the group of rubella seronegative individuals, relative to its frequency in the rubella seropositive group. This association remains statistically significant, at the 5% level, after correction for the number of allele markers typed for. In contrast, HLA-DR5 is seen to be significantly associated with high rubella antibody concentration (remaining so after correction), and associated less strongly with very high antibody levels. The correlation between HLA-DR5 and high rubella antibody concentration remains significant in a subset of female subjects of vaccination age (10-30 years), but not for individuals assumed to be without direct influence of immunization, i.e. excluding females aged between 10 and 30 years.

These last two results seem to imply some disparity in the degree of association of HLA-DR5 with high rubella antibody concentration, according to whether the antibody is vaccine induced or is resultant from natural infection.

Fig. 5.10 illustrates graphically the marked disturbance in the frequency distribution of antibody concentrations in individuals possessing HLA-DR5 (Fig. 5.10a) or HLA-DR4 (Fig. 5.10b) alleles, compared with the overall distribution of concentrations for the 152 JCA patients. A positive shift in the distribution of antibody

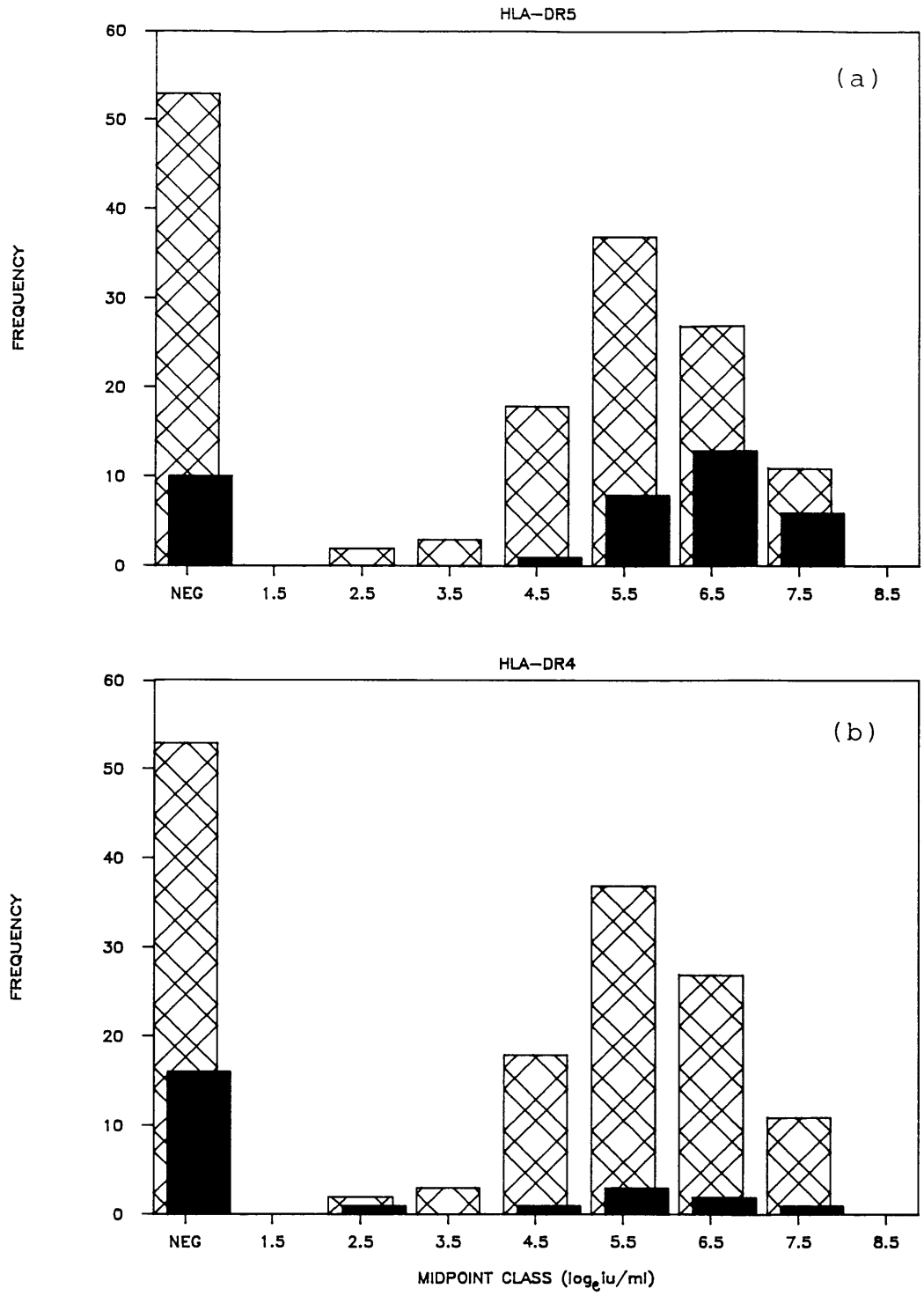


Fig.5.10 Distribution of rubella antibody concentrations for individuals possessing (a) HLA-DR5 or (b) HLA-DR4 from a sample of 152 juvenile chronic arthritis patients (⊠).

concentrations is seen for HLA-DR5 positive individuals, and a preponderance of seronegative results in individuals possessing HLA-DR4.

Any allele which did not exhibit significant association with antibody concentration using the two tail Fisher's Exact test, may show statistical significance in one tail by virtue of a similar association to that of HLA-DR4 or DR5, with which it displays linkage disequilibrium (the phenomenon of preferred allele combinations - see discussion for explanation). Testing samples from each source under this hypothesis one allele satisfied the above criteria. A significant proportion of rubella seronegative individuals from SWBTC possessed HLA-Aw24 ($z=1.75$, $p=4.01 \times 10^{-2}$), an allele which shows strong linkage disequilibrium with HLA-DR4 (see Table 5.9).

No associations between alleles of the HLA-A, B or C loci and rubella antibody were observed for JCA patients.

The relative frequency of ABO blood group phenotypes in volunteer bone marrow donors with either high (>medium) or low positive (<medium) rubella antibody concentration, were compared using a χ^2 test. Data from each source of bone marrow donors for whom blood grouping was available (SEBTC & SWBTC) were analysed separately; neither set of data produced values of χ^2 which were significant at the 5% level (two tail test). The same groups of subjects were analysed with respect to rhesus blood group types, by Fisher's 2x2 Exact test. No significant associations were uncovered. From these results it may, therefore, be inferred that there is no significant association between rubella antibody

concentration and ABO or rhesus blood group markers in the population from which these volunteers were drawn.

(ii) Related samples

The pattern of inheritance of HLA haplotypes in siblings from two family studies (see Table 5.3) have been assessed by the procedure of Green & Woodrow (1977). Prior to analysis, siblings were grouped according to four categories of rubella antibody concntration, HIGH, LOW, VHIGH and VLOW (as defined in Table 5.4). Results for each group are presented in Table 5.8. Observed, S, and expected, μ, scores, and variance, σ^2, are used in calculating the T statistic (see section 5.6). The probability, p, of obtaining each value of T, under the assumption of random haplotype segregation, is determined from normal tables. The null hypothesis may only be rejected if the probability of obtaining a score greater than or equal to that expected, is less than 0.05. Hence the test is one tailed and where the observed score was less that that expected no further analysis was carried out.

No statistcally significant deviation from random assortment of haplotypes was observed for siblings in any of the categories of rubella antibody concentration.

5.9 Discussion

Clear evidence of association between alleles of the HLA-DR

Table 5.8

Association between HLA haplotypes and rubella antibody concentration evaluated by a 'sibling method' of analysis (Green & Woodrow, 1977).

Sample source	Category of [Ab]	Score (S)	Expected Score(μ)	Var(σ^2)	T [2.]	p [3.]
JRH [1.]	HIGH [1.]	24	23.75	5.59	0.106	0.458
	LOW	32	32.31	6.28	-	-
	VHIGH	5	5.50	1.87	-	-
	VLOW	17	16.75	3.84	0.128	0.449
LHW	HIGH	130	133.30	27.26	-	-
	LOW	143	135.81	21.43	1.447	7.4×10^{-2}
	VHIGH	43	43.37	12.84	-	-
	VLOW	52	50.06	11.12	0.429	0.334

1. Abbreviations as defined in Tables 5.2 & 5.4.
2. Normally distributed T statistic, where $T = (S-0.5-\mu)/\sigma$ (see text).
3. Probability, p, of T value occurring by chance (1 tail) from normal tables.

locus and rubella antibody concentration, for patients with juvenile chronic arthritis is demonstrated by this study. HLA-DR5 was observed to be strongly associated with high rubella antibody levels. Analysis of JCA subjects grouped according to vaccination influence (i.e. according to whether or not antibody status could be the result of vaccination) suggests a more pronounced association of this allele with antibody induced by immunization rather than natural infection.

Seronegativity for rubella in JCA patients was found to be highly associated with possession of a HLA-DR4 allele. The association between HLA-DR4, rheumatoid arthritis and susceptibility to rheumatoid factor (RF) production, is well established (Nunez et al, 1984), and the presence of RF or another antiglobulin in serum has been proposed as a possible cause of infrequent false negative rubella RH results in which zone formation is blocked (Champsaur, 1983; Kangro et al, 1983; Truedsson & Sjoholm, 1984). Additionally it is known that RF attaches to the F_c portion of the immunoglobulin molecule (Roitt, 1984), thereby preventing complement binding and inhibiting cell lysis. The possibility of such a 'blocking factor' mechanism accounting for the observed association with HLA-DR4 might be investigated by repeating the rubella serology (negative samples) using a technique which is not dependent upon complement mediated lysis (e.g. the HI test).

It is important to note that exposure to rubella infection for these young individuals who lack specific antibody (average age of 10.3 years) is not assured and this

hinders clear interpretation of the results.

HLA-DR alleles code for class II antigens, present on the cells of the immune system, such as macrophages and B lymphocytes (Dorf (ed.), 1981). These antigens are thought to mediate the cooperation between distinct immune cell populations to control antibody production (Bender, 1984). It is, therefore, not surprising that in this investigation of humoral antibody response the strongest associations uncovered were with these immune response (Ir) genes.

In contrast, HLA-A, B, and C loci code for class I antigens or 'self recognition' markers which are important in cellular immunity to virally infected cells (Bender, 1984). Consequently, it is unlikely that any direct association between these alleles and humoral immunity exists. However, certain HLA allele combinations or haplotypes occur more frequently in a population than would be expected if recombination (crossing over) in chromosome 6 was a random process. Such alleles are said to be in linkage disequilibrium (see Bodmer and Bodmer, 1978, for discussion of this phenomenon). Secondary, weaker associations between HLA-A, B and C alleles and rubella antibody may, therefore, arise as a result of linkage disequilibrium with an Ir gene.

In a study of rubella antibody production, following vaccination, in Japanese recipients, Kato et al (1982) determined a strong association between an allele of the HLA-DR locus (specifically HLA-DRHO, which is not present in caucasian populations) and low antibody concentration. In two other loci the alleles HLA-Aw24 and Bw52 were, again,

statistically higher in low, rather than high, responders, but the significance of these associations was less than for HLA-DRHO. These results were accounted for by the linkage disequilibrium which exists between HLA-Aw24 and Bw52 and the HLA-DRHO alleles.

No associations between alleles coding for class I antigens and rubella antibody levels were uncovered for JCA patients. This may be due to the relatively small sample size or as a consequence of the assumed low proportion of seronegative individuals exposed to rubella infection. Both of these limitations were less critical for the surveys of adult unrelated volunteer bone marrow donors from two sources in England. Analysis of phenotype frequencies revealed a number of weak associations between HLA-A and B alleles and rubella antibody concentration. It is possible that these associations are a result of linkage disequilibrium HLA-DR alleles more directly associated with humoral immune response. No HLA-DR typing was available for the bone marrow volunteers.

HLA-A2 and A3 were found to be associated with high antibody concentration in samples from SEBTC. These two alleles exhibit linkage disequilibrium with HLA-DR5 (see Table 5.9) an allele which was strongly associated with high rubella antibody levels in JCA patients. Employing the one tailed Fisher´s Exact test, HLA-Aw24 was determined to be associated with seronegativity in adults from SWBTC. The same association was revealed for HLA-DR4 in JCA samples, an allele which is in linkage disequilibrium with HLA-Aw24 (Table 5.9). These results suggest the involvement of Ir

Table 5.9

The most frequently observed allele combinations of HLA-A, B and C with HLA-DR4 or DR5 in caucasians (from Bender, 1984).

Haplotype				Frequency
A2	B12	Cw5	DR4	0.0177
A2	B15	Cw3	DR4	0.0126
A3	B7	Cw-	DR5	0.0116
A2	B12	Cw-	DR5	0.0101
A1	B17	Cw-	DR5	0.0094
Aw24	B15	Cw3	DR4	0.0088
A3	B15	Cw3	DR4	0.0085

genes in rubella antibody response that are common to young and adult individuals. A follow-up study on HLA-DR typed unrelated normal adults would be of considerable interest.

It should not be assumed that the strong associations between HLA-DR4 or -DR5 and rubella antibody concentration for samples from CRC, will hold for the population in general. Altered HLA phenotype frequencies for patients with pauciarticular early onset juvenile chronic arthritis (the particular form of the disease most of the patients in this study suffer from) compared with normal controls, illustrates this point (see Hall et al, 1986, for further details on this hetrogeneous disorder). It is also of interest that two previous investigations, one of UK vaccinees (Harcourt, Best & Banatvala, 1979) and the other of a Danish population (Hansen, Larsen & Leehoy, 1980), reported a complete absence of correlation between HLA antigens and rubella antibody. Further doubts are raised by the results of the two family studies, neither of which produced evidence of deviation from random assortment of haplotypes in siblings grouped according to rubella antibody concentration. This was particularly surprising for the large RA study because the haplotypes included HLA-DR locus alleles.

The associations between HLA alleles and rubella antibody concentration derived from data from SEBTC and SWBTC are tentative and caution should be applied in their interpretation. The low confidence in these associations stems, not so much from the low statistical significance of the individual correlations (none were significant when

corrected for the number of specificities typed for), but from the lack of corroborative evidence between the two surveys, i.e. associations from one survey were not confirmed by the other. In addition, none of the results correlate with HLA associations uncovered in vaccinated American caucasians (Spencer et al, 1977) or in naturally immune inhabitants of the Orkneys (Poskanzer et al, 1980).

It should be pointed out that confidence in the experimental reproducibility was good and samples of bone marrow donors were considered to be representative collections of adult, unrelated caucasians in the population. Therefore, the results for SEBTC and SWBTC are likely to be a true reflection of the situation in this country.

The results of this study provide additional evidence that HLA determinants are involved in the immune response to rubella virus. The possibility of a genetic association with seronegativity or with the rate of antibody decay, which influences the shape of age-serological profiles (perhaps accounting for the less than 100% immunity in the older age classes), raises doubts about the accuracy of the age-dependent rates of infection derived from such data (Anderson and May, 1985a). The importance of accurate estimates of transmission rates to the outcome of mass vaccination campaigns is considered in the following chapter.

Chapter 6

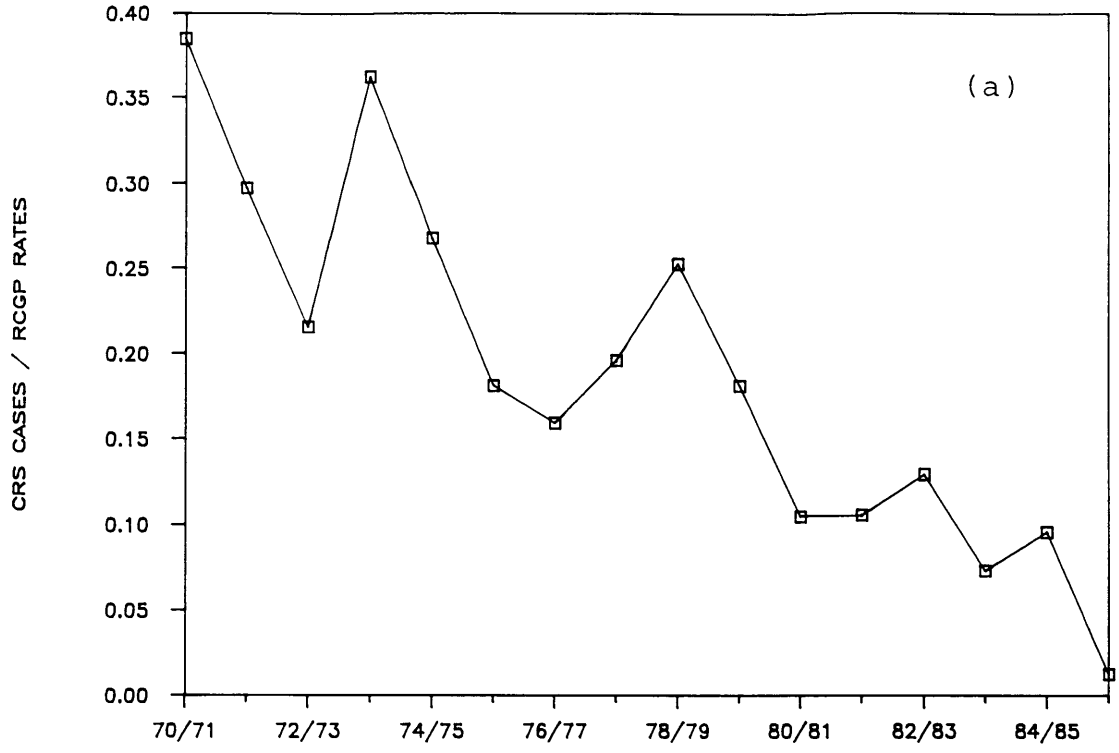
RUBELLA VACCINATION POLICIES IN THE UNITED KINGDOM: PREDICTIONS OF FUTURE TRENDS

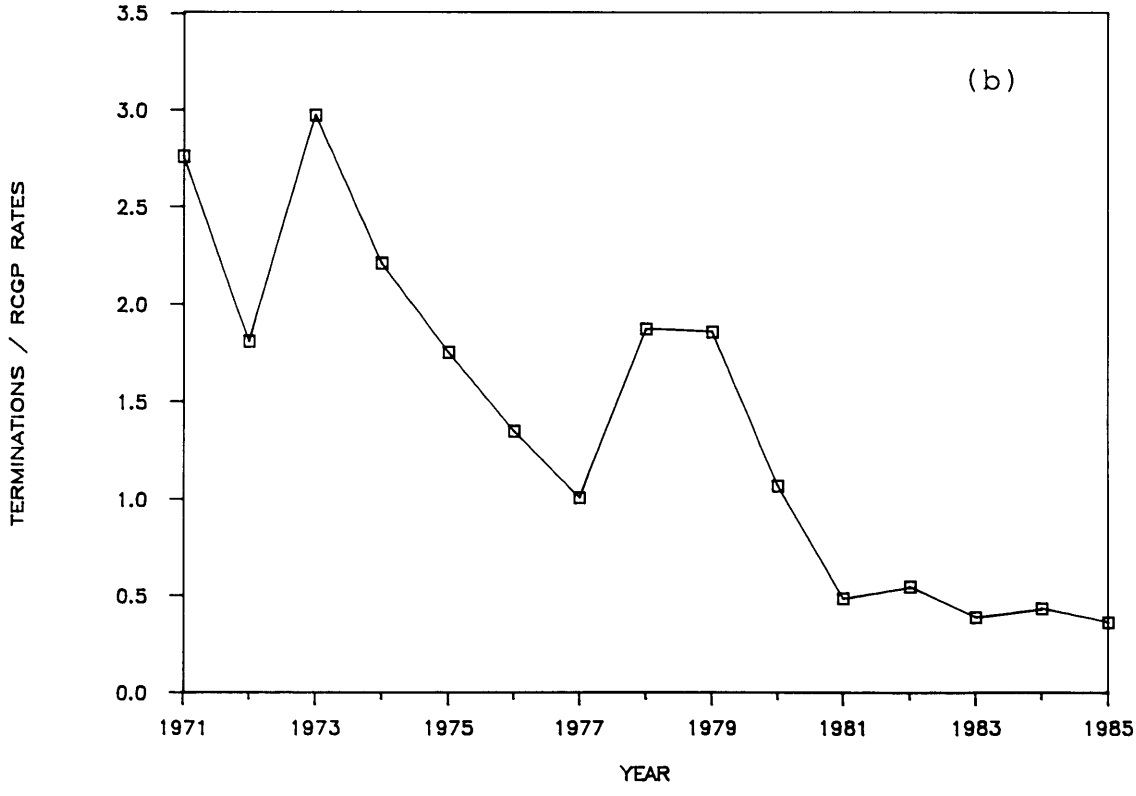
6.1 Introduction

Considerable uncertainty surrounds the future of rubella vaccination policy in the United Kingdom. The current programme of schoolgirl and adult seronegative female immunization has resulted in a marked decline in the incidence of congenital rubella, and rubella associated terminations of pregnancy (Fig. 6.1a & b); trends which are likely to continue for some time (see section 3.4). Nevertheless, there is concern that such reductions are inadequate (Miller et al, 1985; Banatvala, 1985). The realisation that selective immunization is unlikely to achieve total elimination of congenital rubella (because it requires 100% immunization of females prior to entering the childbearing age classes) has been emphasised by the success, in this respect, of the US mass childhood immunization programme (Bart et al, 1985a,b; Report, 1986).

Much debate is now in progress on whether to continue with the current policy in this country (encouraging higher levels of selective immunization, particularly of

Fig. 6.1. Decline in CRS cases and rubella associated terminations since 1970 accounting for fluctuations in the incidence of postnatal rubella. Graph (a) records the number of cases of CRS in England from July to June of each year over the interval 1970-85 (reported to NCRSP Northern and Southern registries) divided by the corresponding annual RCGP rate (per 100,000 population) of rubella incidence (source: Dr.N. Noah, CDSC) over the same period. Graph (b), in a similar way, shows the changes in the annual number of therapeutic abortions carried out in England and Wales as a result of rubella infection during pregnancy (source: OPCS Ab series, 1986), scaled by the incidence of acquired rubella. Similar trends result from using laboratory reports of rubella (annual figures from CDSC) in place of RCGP rates.


(a)
CRS CASES / RCGP RATES
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
70/71
72/73
74/75
76/77
78/79
80/81
82/83
84/85


(b)
TERMINATIONS / RCGP RATES
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1971
1973
1975
1977
1979
1981
1983
1985
YEAR

seronegative women in the 'at-risk' age groups), or to implement a combined UK and USA strategy, i.e. selective and childhood immunization, in order to reduce the circulation of wild virus in the population (Miller & Miller, 1985; Anderson & Grenfell, 1985).

A reluctance to proceed with a dual-stage policy is partly a result of disquieting theoretical evidence which suggests that low levels of cohort immunization (boys and girls) can result in more cases of rubella infection in older age classes (and subsequently more cases of congenital rubella) than that pertaining prior to the introduction of mass vaccination (Knox, 1980; Anderson & May, 1983). This is supported by recent empirical observation for rubella in the USA (Bart et al, 1985a; CDC, 1984). Any benefit to be gained from a mixed UK and USA policy over selective immunization alone is thought to be dependent upon what vaccination coverage of male children is achievable (Anderson & May, 1983). At present, this is an unknown quantity in this country.

Amongst all this uncertainty, however, there is some agreement that decisions on the choice of modifications to the UK policy should be made in the light of theoretical predictions of the effectiveness of such programmes. In a recent publication, Anderson and Grenfell (1986) addressed this issue. A mathematical model of rubella virus transmission was used to simulate the relative impact of a number of alternative policies, based on either a selective or a combined strategy (see Table 6.2) on the incidence of CRS. Two important features of the model were thought to

give improved realism to their predictions, compared with previous studies. Firstly, it incorporated information on age-dependent rates or forces of infection. Force of infection estimates were obtained from case notification data and from serological surveillance (those for males from the survey of S.E. England presented in Part 1 of Chapter 3). Secondly, the model took into consideration heterogeneity in mixing within- and between-age groups.

The principal conclusions from their study were that (a) the most effective immunization strategy is dependent upon the nature of age-related changes in the force of infection and (b) on the basis that the most accurate force of infection estimates are derived from serological (as opposed to case notification data) then levels of vaccination of 2 year old boys and girls must exceed 60% to obtain significant benefit from a combined UK/USA policy, over that derived from selective immunization alone.

Anderson and Grenfell (1986) expressed two reservations about making definite suggestions on which policy to adopt for the future in the UK. The first was the paucity of epidemiological information for rubella from which to estimate age-dependent transmission rates, and the lack of knowledge to determine the accuracy of this information. The second was that if it is assumed that rubella vaccine uptake in children will mirror that for measles (at that time around 63% nationally) then the case for choosing a combined policy is a borderline one.

The present study forms a direct continuation of this work of Anderson and Grenfell, in an attempt to obtain a

clearer impression of the optimal rubella vaccination strategy for the United Kingdom. The investigation is pursued along a number of themes:

(i) To further study the dependence of predictions upon age-related rates of infection utilising the more expansive epidemiological database for rubella now available (presented in Chapters 3 and 4). In this context the analyses of case notifications (Chapter 4) and of quantitative serological data (Chapter 5) should allow a more informed interpretation of the estimated forces of infection.

(ii) To look at recent trends in vaccination rates for rubella in teenage girls and for measles in children, and in age-specific fertility rates, to determine their importance to the choice of vaccination strategy.

(iii) In view of the additional cost of maintaining both selective and mass childhood immunization (were a mixed policy implemented), to evaluate the possibility of dispensing with the selective stage at some time in the future.

Initially an outline is given of the mathematical model employed to make the predictions, together with sources of empirical data and the method of presentation of results.

6.2 Mathematical Model of Rubella Virus Transmission

A compartmental deterministic model of rubella virus transmission in a large age- and sex-structured community is used to examine the impact of different vaccination policies on the incidence of CRS. The structure of this model is described in detail in a paper by Anderson and May (1985a), and its utilization for the purposes of this present study closely follows the procedures adopted by Anderson and Grenfell (1986). The following provides only a summary of the model structure and assumptions, and description of the method for presentation of model predictions. Therefore, the reader is referred to the above two publications for more formal and comprehensive accounts.

(i) Model structure and parameter estimates

The model considers a population of constant size (where the net birth rate exactly balances the net death rate) in which individuals are categorized according to rubella infection status (the infection is assumed to be endemic). The number (or density) of individuals of age a and sex k (k = 1, 2 where 1 = male, 2 = female) at time t, who are protected by maternal antibodies, susceptible, infected but not infectious (latent), infectious, and immune (via natural infection or vaccination), are defined by the variables $M_k(a,t)$, $X_k(a,t)$, $H_k(a,t)$, $Y_k(a,t)$ and $Z_k(a,t)$ respectively. Immunity is assumed to be of lifelong duration (acquired either by infection or vaccination).

The rates of change of these variables with respect to both age a and time t may be expressed as a set of non-linear partial differential equations;

$$\frac{\partial M_\kappa(a,t)}{\partial t}+\frac{\partial M_\kappa(a,t)}{\partial a}=-[\mu(a)+d]\,M_\kappa(a,t). \tag{1}$$

$$\frac{\partial X_\kappa(a,t)}{\partial t}+\frac{\partial X_\kappa(a,t)}{\partial a}=dM_\kappa(a,t)-[\mu(a)+\lambda(a,t)+v(a,t,\kappa)]\,X_\kappa(a,t). \tag{2}$$

$$\frac{\partial H_\kappa(a,t)}{\partial t}+\frac{\partial H_\kappa(a,t)}{\partial a}=\lambda(a,t)\,X_\kappa(a,t)-[\mu(a)+\sigma]\,H_\kappa(a,t). \tag{3}$$

$$\frac{\partial Y_\kappa(a,t)}{\partial t}+\frac{\partial Y_\kappa(a,t)}{\partial a}=\sigma H_\kappa(a,t)-[\mu(a)+\gamma]\,Y_\kappa(a,t). \tag{4}$$

$$\frac{\partial Z_\kappa(a,t)}{\partial t}+\frac{\partial Z_\kappa(a,t)}{\partial a}=\gamma H_\kappa(a,t)+v(a,t,\kappa)\,X_\kappa(a,t)-\mu(a)\,Z_\kappa(a,t). \tag{5}$$

Here, $\mu(a)$ is the age-specific mortality (set to zero up to life expectancy L which is assumed to be 75 years, and infinite thereafter), 1/d is the average duration of protection provided by maternally derived antibody (approximately 0.25 years; determined in Section 3.2), $1/\sigma$ is the latent period (10.5 days) and $1/\gamma$ is the period of infectiousness (11.5 days) (Hanshaw & Dudgeon, 1978; Anderson & May, 1983). The age-, time- and sex-dependent rate of vaccination is represented by v(a,t,k). Estimation of the rates of vaccination are considered in the next subsection.

The per capita rate or force of infection, $\lambda(a,t)$, is defined as

$$\lambda(a,t)=\int_0^L \beta(a',a)\sum_{i=1}^{2} Y(a',t)\,da' \tag{6}$$

where Y(a',t) denotes the density of infectious people of age a at time t, and the term $\beta(a',a)$ is the transmission

coefficient arising from contact of susceptibles of age a with infectious individuals of age a'. The per capita rate at which susceptibles of age a at time t are infected, $\lambda(a,t)$, is, therefore, the sum over all ages of the product of the transmission coefficient, $\beta(a',a)$, times the number of infectious individuals, $Y(a',t)$, of both sexes ($k = 1,2$). In simple terms, the force of infection is a function of the rate of contact between susceptibles of one age and infectious individuals of all ages, which incorporates the heterogeneity in, both, the age distribution of infectious people, and, the degree of interaction (contact and transfer of infection) between individuals of differing age, which exists in a community.

The force of infection is of central importance to the accuracy with which equations (1)-(5) model the transmission dynamics of rubella infection in a population. However, its estimation via equation (6) poses some difficulty because accurate determination of $\beta(a',a)$, the transmission coefficient, from epidemiological observation, is not straightforward (unlike the parameters d, σ & γ).

Any given value of the transmission coefficient, $\beta(a'a)$, represents the rate of contact between susceptibles of one particular age, a, and infectious individuals of another age, a', and, the likelihood that such an interaction will result in a new case of rubella infection (a function of the innate contagiousness of the aetiological agent and/or the susceptibility of the host). The phrase 'who acquires infection from whom' has been coined by Anderson and May (1985a) to describe this term $\beta(a',a)$.

To simplify estimation of the transmission coefficient, the age range is divided into a number of discrete age classes, i = 1, 2, .. n, such that the term β_{ij} represents the coefficient for rubella transmission between susceptibles of age class i, and infectious individuals of age class j. The age-specific forces of infection, λi^* (where i = 1, 2, .. n) are then related to the β_{ij} coefficients in the manner

$$\lambda_i = \sum_{j=1}^{n} \beta_{ij} \bar{Y}_j \qquad (7)$$

here $\bar{Y}$ denotes the total number of infectious people of age class j.

The set of β_{ij}'s obtained from all the possible combinations of susceptible and infectious age classes (numbering i.j) form a two-dimensional array known as a 'who acquires infection from whom' matrix (shortened to WAIFW matrix). The value of each β_{ij} may be calculated from age-specific forces of infection, λi, using equation (7), under the condition that the number of unique values of β_{ij} does not exceed the number of age classes for which λi values have been estimated (see Anderson & May, 1985a, for mathematical constraints on the model). The age-dependent forces of infection derived in Chapters 3 and 4 from the serological surveys of S.E. England and S. Yorkshire and from the case notifications for the city of Bristol, provide various sets of λi^* estimates which are employed in this study.

More than one arrangement of the β_{ij} values in a

* Notice here that λi replaces $\lambda(a)$ for mathematical convenience only.

particular WAIFW matrix will satisfy a set of λi estimates from which they are derived. In other words there are a number of configurations of a set of β_{ij}'s (each describing different degrees of mixing and infection transfer in a population) that will generate an observed set of age-dependent forces of infection.

However, Anderson & Grenfell (1986) concluded that the predicted impact of various vaccination policies on CRS incidence was relatively insensitive to the arrangement of the β_{ij} values in a WAIFW matrix. Consequently, a single WAIFW matrix configuration is considered in this study:

Age group	1	2	3	4	5
1 (0–5 years)	β_1	β_1	β_3	β_4	β_5
2 (5–10 years)	β_1	β_2	β_3	β_4	β_5
3 (10–15 years)	β_3	β_3	β_3	β_4	β_5
4 (15–20 years)	β_4	β_4	β_4	β_4	β_5
5 (20–75 years)	β_5	β_5	β_5	β_5	β_5

WAIFW1

5 age classes are chosen which accommodate the λi estimates for serology and case notifications shown in Tables 3.2 & 4.1. Note, therefore, that it is possible to have only 5 distinct β_{ij} values.

The arrangement of coefficients in WAIFW1 is not arbitrary, but reflects the importance of within school interactions between children. It is assumed that mixing within age classes is most pronounced in the children attending school (hence the unique β_2 value for the 5-9 year

olds), with high degrees of mixing within- and between-young children (0-4 years) and young teenagers (10-14 years), denoted by β_1 and β_3 respectively. The rate of mixing within- and between-age classes is assumed to be similar over the age band 15-75 years.

(ii) Immunization policies and rates of vaccine coverage

The model takes explicit account of the age-, time- and sex-dependent rate of rubella vaccination, v(a,t,k) (see equations (2) and (5)). Table 6.1 shows the actual proportion of girls in each yearly cohort in England and Wales, vaccinated by age a, for the years 1970-1984. These are determined from DHSS vaccination records (SBL607/1985) and OPCS age-stratified population estimates (PP1 86/1). The figures shown in Table 6.1 are used in the rubella transmission model to simulate the impact of the first 15 years of the UK selective immunization programme.

From 1985 to 2050 any of four alternative vaccination policies are put into effect. These are shown in Table 6.2, and broadly represent the options for either a continuation of selective female vaccination (policies 1 and 3) or a combined selective and mass childhood vaccination campaign (policies 2 and 4). The rate of vaccination of teenage girls for 1985 onwards is assumed to remain unchanged from 1984 levels (these are the latest figures available). In absence of accurate national estimates for the rate of immunization of adult seronegative women in this country, an arbitrary rate of 50% of 25 year old females is adopted, for

Table 6.1

Percentages of schoolgirls (not previously vaccinated), of different ages, immunized against rubella in England and Wales from 1970 to 1984 (determined from DHSS and OPCS statistics, see text)

	Age (years)					
Year	10	11	12	13	14	15
1970		9.77	13.32	9.15		
1971		30.40	47.67	33.64		
1972		22.31	44.72	46.37		
1973		19.23	34.60	48.41		
1974		18.07	30.65	33.85		
1975		18.35	30.97	30.92		
1976		20.94	34.09	33.87		
1977		23.36	36.60	30.46		
1978		22.53	38.90	32.50	12.64	
1979	0.82	23.53	44.72	41.12	18.68	5.09
1980	0.75	25.45	42.65	40.28	17.66	6.59
1981	1.30	25.37	45.93	38.90	15.53	5.25
1982	3.50	29.60	45.20	40.24	14.54	4.93
1983	5.64	29.96	46.67	41.10	15.31	3.94
1984	7.54	31.03	49.39	42.14	16.35	5.32

Table 6.2

Alternative rubella vaccination policies (Anderson & Grenfell, 1986)

Type of policy	Description
Single-stage (policy 1)	A UK policy in which only girls are vaccinated between the ages of 10 and 15 years
Dual-stage A (policy 2)	A combined UK and USA policy in which girls and boys are vaccinated at 2 years of age, and girls at between 10 and 15 years of age
Dual-stage B (policy 3)	A UK policy plus the vaccination of susceptible women of 25 years of age
Triple-stage (policy 4)	A combined UK and USA policy plus vaccination of susceptible women of 25 years of age

policies 3 and 4. The intention is to obtain a qualitative, rather than a quantitative, assessment of the impact of this stage in either policy. Finally, various levels of vaccination of 2 year old boys and girls are considered for policies 2 and 4, although as a basis for most simulations it is assumed that the proportion vaccinated mirrors that currently achieved for measles vaccine (i.e. 68% in 1985).

(iii) Presentation of model predictions

The predicted incidence of CRS at any time, t, after the initiation of a vaccination programme divided by the average CRS incidence prior to vaccination, provides a convenient measure of the effectiveness of a given rubella immunization policy. This relationship is embodied in the term $w(a_1,a_2,t)$, called the CRS ratio, which is defined as

$$w(a_1,a_2,t) = \frac{\int_{a_1}^{a_2} m(a) X(a,t) \lambda(a,t)}{\int_{a_1}^{a_2} m(a) X(a,0) \lambda(a,0)} \qquad (8)$$

where X(a,t) denotes the number of female susceptibles at time t of age a, and $\lambda(a,t)$ represents the age- and time-dependent force of infection. Time-dependent solutions of these two variables are provided by the model of rubella transmission. The function m(a) defines the probability or risk of a susceptible women of age a being pregnant. This risk function is derived from age-dependent fertility data between the ages of 16 and 40 years (a_1 and a_2, respectively, which delimit the childbearing ages) provided

by OPCS, FM1 No.11, 1985.

The ratio, $w(a_1,a_2,t)$, therefore, is an estimate of the total number of cases of acquired rubella in pregnant women (each, potentially, resulting in a case of CRS) at time t after vaccination (numerator in equation (8)), divided by the number of potential CRS cases before vaccination (denominator in equation (8)).

Temporal changes in the CRS ratio, under the impact of vaccination programmes detailed in Table 6.2, are presented in the results, together with the long-term benefit of a given policy (which is estimated from the average CRS ratio over the years 1985 to 2050). Additionally, model predictions of the proportion of individuals of either sex who are in the immune class, are plotted against time and age to produce 'serological surfaces' for a population.

6.3 Model Predictions of CRS Incidence: Influence of Various Factors

(i) Age-dependent infection rates

Age-dependent rates of rubella transmission derived in Chapters 3 & 4 from horizontal, cross-sectional serological and case notification data (various sources in England), are summarized in Fig. 6.2 (see also Tables 3.2 & 4.1). Pre-vaccination estimates of the forces of infection (male and female pooled), and estimates from data collected since initiation of vaccination in 1970 (male only), are

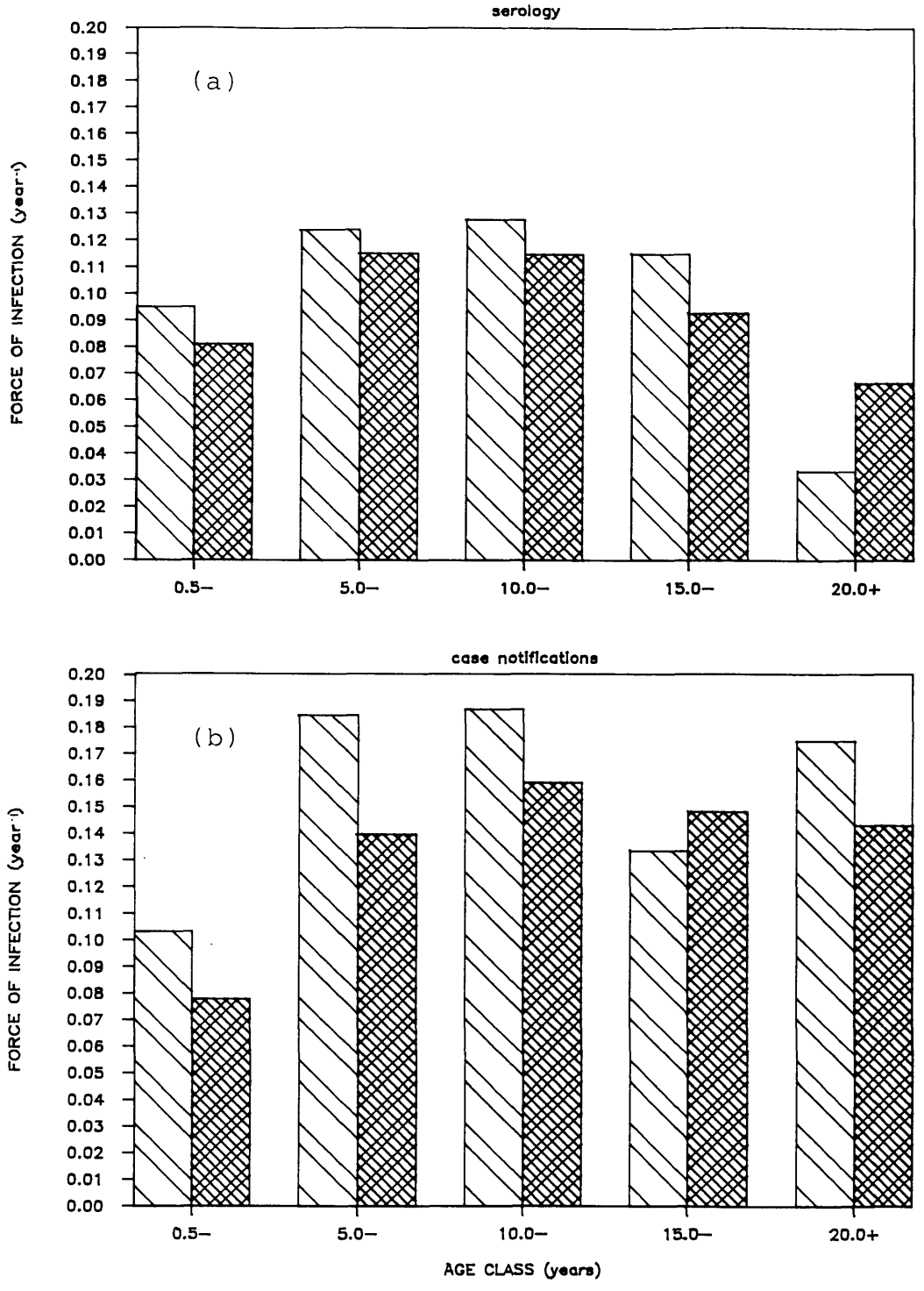


Fig.6.2 Age-dependent forces of infection for rubella: various sources. Estimates of the rate of rubella transmission for specified age classes were derived, in the top graph, (a), from the serological surveys of S. Yorkshire (male and female data) 1969, (▨), and of S.E. England (male data only) 1980/84, (▩), and in the bottom gragh, (b), from case notifications for the city of Bristol, either males and females pooled from the years 1967-69 (▨) or males only 1978-1983 (▩). See Tables 3.2 and 4.1.

represented in each graph (Fig. 6.2a & b).

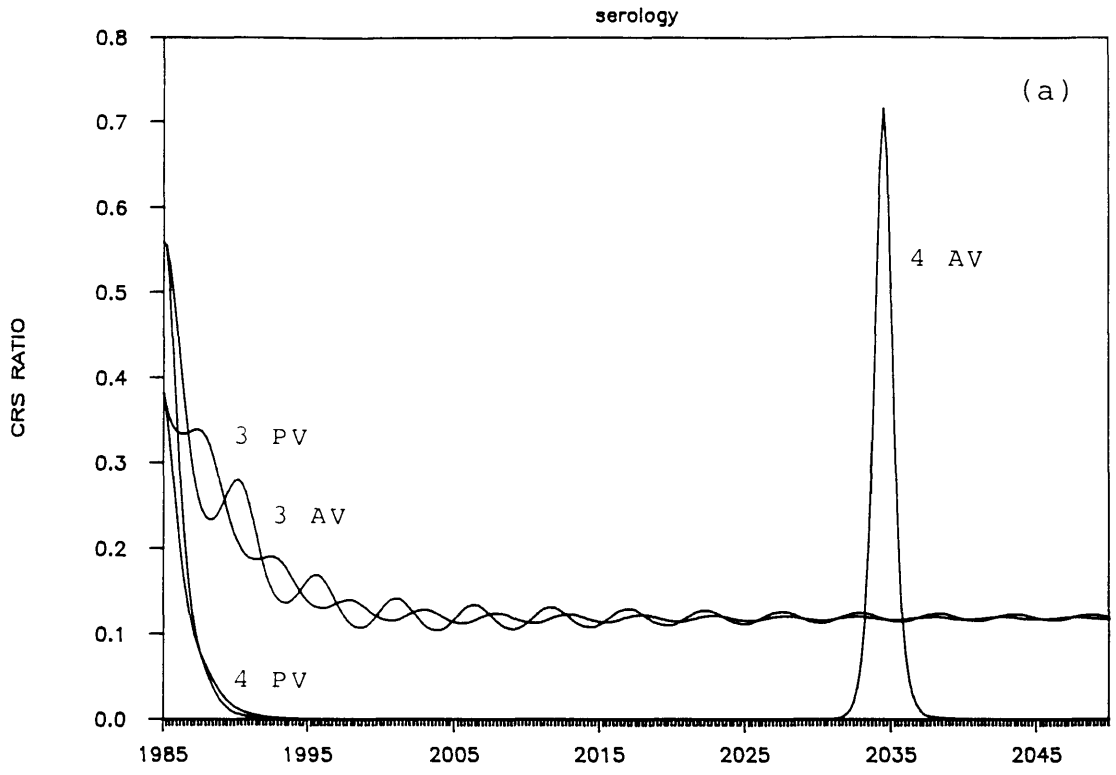
A high degree of similarity between pre- and post-immunization infection rates is exhibited by both types of epidemiological information, i.e. serology in Fig. 6.2a and case notifications in Fig. 6.2b. This suggests that transmission of rubella in the male commmunity has been largely unaffected by the selective immunization programme in the UK. However, the $\lambda(a)$ values derived from serology differ markedly from those for case notifications both in magnitude and in the pattern of change, with age (compare Fig. 6.2a with Fig. 6.2b). Estimates of the force of infection from serological data are, in general, lower, and show a more obvious decline into the adult age classes (20 years and over).

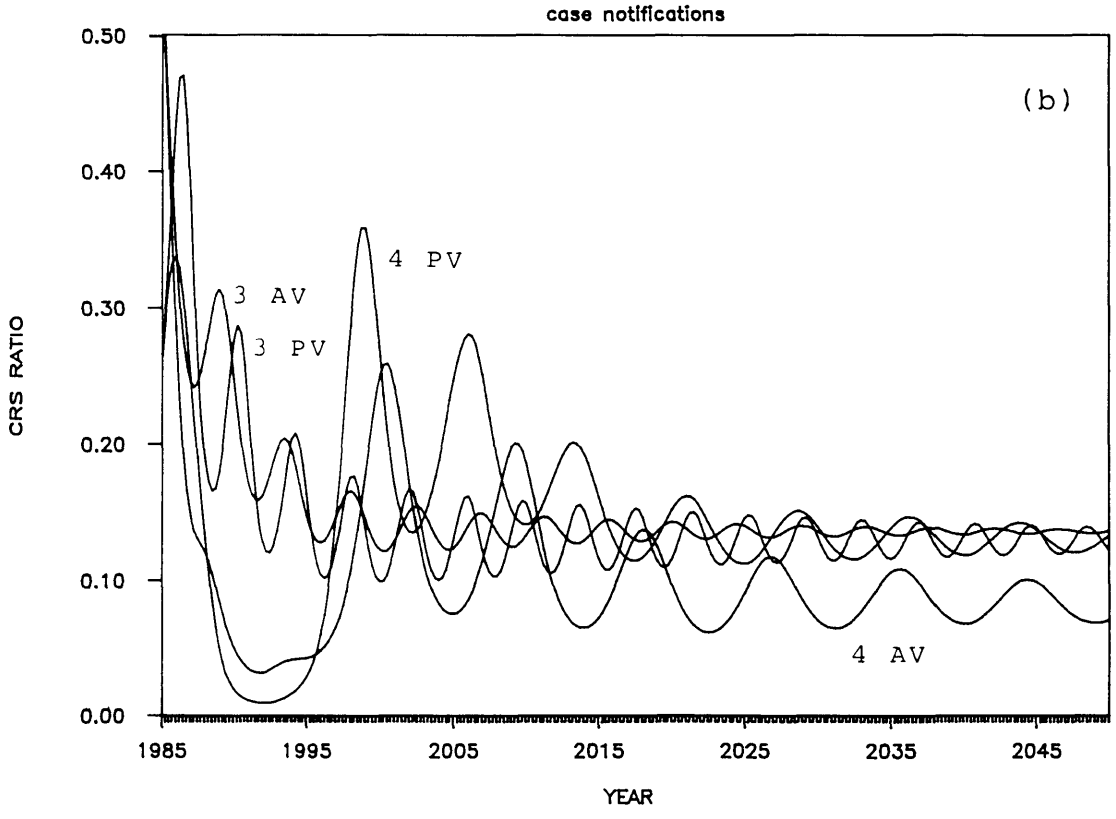
The nature of age-related changes in the force of infection within a community is of considerable importance to the predicted impact of different rubella vaccination policies on the incidence of CRS in a community (Anderson & Grenfell, 1986). This point is clearly illustrated in Fig. 6.3. Each graph represents the simulated outcome of a two-stage selective policy and a combined UK and USA programme of vaccination (policy 3 and 4, respectively, from Table 6.2) on CRS incidence.

The predictions in the top gragh (Fig. 6.3a) are based on $\lambda(a)$ estimates derived from serology (Fig. 6.2a) and those in the bottom gragh (Fig. 6.3b) are based on estimates of the rates of transmission derived from case notification records (Fig. 6.2b).

The influence of each policy on the incidence of CRS is

Fig.6.3 Age-dependent forces of infection for rubella: influence on model predictions of the incidence of CRS. Changes in the CRS ratio, $w(a_1,a_2,t)$, (i.e. the number of potential congenital rubella cases over a short time period after initiation of vaccination divided by the average number of CRS cases over the same period of time prior to immunization) are recorded with successive intervals of time after vaccination. Simulations in the top gragh, (a), are based on estimates for the age-related changes in rubella transmission derived from pre-vaccination serology (PS) and serology after onset of vaccination (AS) (see Fig. 6.2a), whereas the simulations in the lower gragh, (b), are based on $\lambda(a)$ estimates from pre- (PC) and post- (AC) vaccination case notification data (see Fig. 6.2b). The predicted impact of two vaccination strategies, labelled 3 (selective policy 3) and 4 (combined USA/UK policy 4) in this figure (and detailed in Table 6.2) have been investigated for each of the four sources of $\lambda(a)$ estimates. These alternative programmes are implemented in 1985 following 15 years of single-stage, selective immunization of schoolgirls (policy 1) since 1970 (levels of vaccination for each stage of the two policies are given in the text). Note that in 1970 the CRS ratio was equal to unity.


serology
(a)
CRS RATIO
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
4 AV
3 PV
3 AV
4 PV
1985
1995
2005
2015
2025
2035
2045


case notifications
(b)
CRS RATIO
0.50
0.40
0.30
0.20
0.10
0.00
4 PV
3 AV
3 PV
4 AV
1985
1995
2005
2015
2025
2035
2045
YEAR

measured as the number of potential CRS cases over a short time interval after the initiation of vaccination, divided by the average number of predicted cases of CRS, over the same time interval, prior to immunization (abbreviated to CRS ratio)(See Section 6.2(iii)). The expected number of CRS cases in any one time interval is calculated from the number of cases of rubella infection in females aged between 16 and 40 years (the ages in which 99% of all pregnancies occur in the UK) and the probability that a female of a particular age, who contracts rubella, is pregnant at the same time (this is a function of age-specific fertility - see next subsection)(Anderson & May, 1983).

The two policies investigated are the most likely alternative future vaccination programmes for this country. Their implementation is assumed to begin in 1985; this follows 15 years of single stage selective immunization of teenage girls (see Table 6.1). In the generation of the predictions between 1985 and 2050 the proportion of each age class vaccinated was held constant at (i) 1984 levels for teenage girls (see Table 6.1), (ii) 50% of seronegative women at 25 years of age, and (iii) 68% of boys and girls at age 2 years (policy 4 only).

The outcome of selective vaccination of teenage girls and adult seronegative women (policy 3 in Fig. 6.3a & b) is unaffected by the source of $\lambda(a)$ estimates and, hence, is independent of the pattern of age-related changes in rubella transmission rates. The reduction in the incidence of CRS after initiation of the policy is directly proportional to the reduction in the number of seronegative females entering

the childbearing ages as a result of immunization.

Only when there is a change in the age-distribution of susceptibles in the community, such as would be achieved by the mass vaccination of boys and girls at a young age, will age-related changes in the force of infection significantly influence the outcome of an immunization programme (Anderson and May, 1985a,b; Anderson and Grenfell, 1986).

Mass childhood immunization restricts the number of infectious individuals in the young age classes, thereby substantially reducing the overall rate of virus transmission in the community. As a consequence of this, following the implementation of a combined UK/USA programme (policy 4), there is an immediate decline in the CRS ratio over that predicted to exist under a continued selective vaccination policy only (Fig. 6.3a & b).

However, the tendency of mass vaccination to raise the average age at infection, shifts the remaining susceptibles (who escaped immunization and infection) into the older age classes. This can result in an increased proportion of seronegative females of childbearing age over that pertaining prior to the introduction of childhood vaccination. Analysis of case notification records suggests little change in the force of infection with increasing age (Fig. 6.2b). Thus the probability of these susceptible women, in the 'at-risk' years, acquiring rubella infection remains relatively high, and in consequence, the CRS ratio is expected to rise once more, such that a selective or a combined vaccination programme (policy 3 and 4, respectively) are similarly effective in the long run

(Fig. 6.3b).

Force of infection estimates derived from serological data indicate a considerably lower probability of acquiring rubella infection in the adult age classes (Fig. 6.2a). Therefore, mass vaccination will tend to displace the remaining susceptible females from an age class with a high force of infection into an age class with a relatively low infection rate. As a result of this, until the number of susceptible individuals builds up to epidemic proportions, the incidence of rubella infection, and therefore of CRS, will remain low (Fig. 6.3a).

Reduction in the rate of infection in a community, caused by childhood vaccination, increases the critical number of susceptibles required for an epidemic (the principle of herd immunity; Anderson & May, 1983), and therefore increases the interepidemic period (compare predictions for policy 4 with those for policy 3 in Fig. 6.3a & b). Furthermore, the upward shift in the age-distribution of susceptibles, under mass vaccination, means that the force of infection in the older age classes is of particular importance in determining to what degree the interepidemic period is lengthened. A trend may be seen in the $\lambda(a)$ estimates derived from case notifications (Fig. 6.2b), post-vaccination male serology, and pre-vaccination serology (Fig. 6.2a), in the extent to which the force of infection declines into the adult age classes. Consequently, the predictions shown in Fig. 6.3a & b, based on these sources, show a concomitant trend for change in the interepidemic period under policy 4.

In the following subsections ((ii) - (v)) model predictions are based on the age-dependent forces of infection for males derived from the serological survey of S.E. England 1980/84 (Fig. 6.2a). These $\lambda(a)$ estimates, as opposed to those derived from Sheffield serology or case notification data (Fig. 6.2), are considered to most accurately represent rubella transmission rates in England in the absence of mass vaccination. Justification for such a conclusion is discussed in section 6.4.

(ii) Trend in age-specific fertility

Since initiation of the rubella vaccination policy in 1970, there has been an increase in the number of births to older women (30 years and over) and a decline in the birth rate in younger women (under 20 years), resulting in an overall upward shift in age-specific fertility in this country Fig. 6.4).

Such a change is likely to have a measurable effect on the incidence of CRS; an older average age of pregnancy in women means a greater probability that females will be infected with rubella (and therefore immunized), before entering the childbearing ages. This results in a concomitant reduction in CRS cases. In other words over the last ten years the probability of a female having a primary rubella infection whilst pregnant has declined.

The outcome of the changes in age-related fertility, shown in Fig. 6.4, on the number of cases of CRS is, however, predicted to vary according to the implemented

Fig.6.4

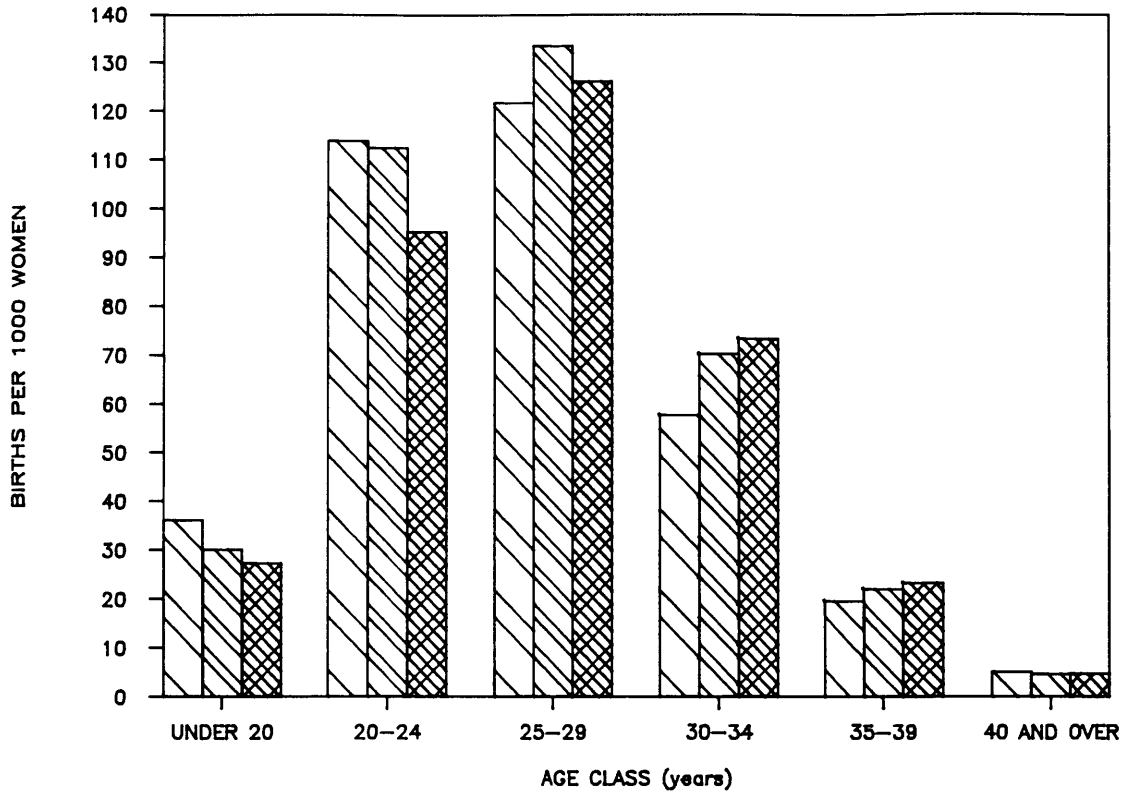


Fig.6.4 Changes in age-specific fertility rates between 1975 and 1985 from figures for England and Wales provided by the Office of Population Censuses and Surveys (OPCS FM186/2, 1986). Note the overal trend for increase in the average age of women at childbirth.

Fig.6.5 Age-specific fertility trend, 1975-1984: effect upon CRS incidence under different vaccination policies. The gragh is similar to that described for Fig. 6.3. However for each vaccination strategy (i.e. policy 3, 3, and policy 4, 4) the simulation is repeated using a different risk function (i.e. the probability that a female of age a who contracts rubella is pregnant, m(a)) calculated from age-specific fertility data for the two extreme years shown in Fig. 6.4 (using more finely age-stratified figures from OPCS cohort series FM1/11); these are labelled 1975 and 1984. Predicted changes in the ratio w(a a t) are based on age-dependent forces of infection derived from S.E. England male serology (see Fig. 6.2a).

Fig.6.6 Age-specific fertility trend, 1975-1984: long-term benefit. The histogram shows the average value of the CRS ratio over the years 1985 to 2050 (i.e. an indication of the long-term benefit of an immunization programme) calculated from each simulation recorded in Fig. 6.5. (labels are as described for Fig. 6.5).

Fig.6.5

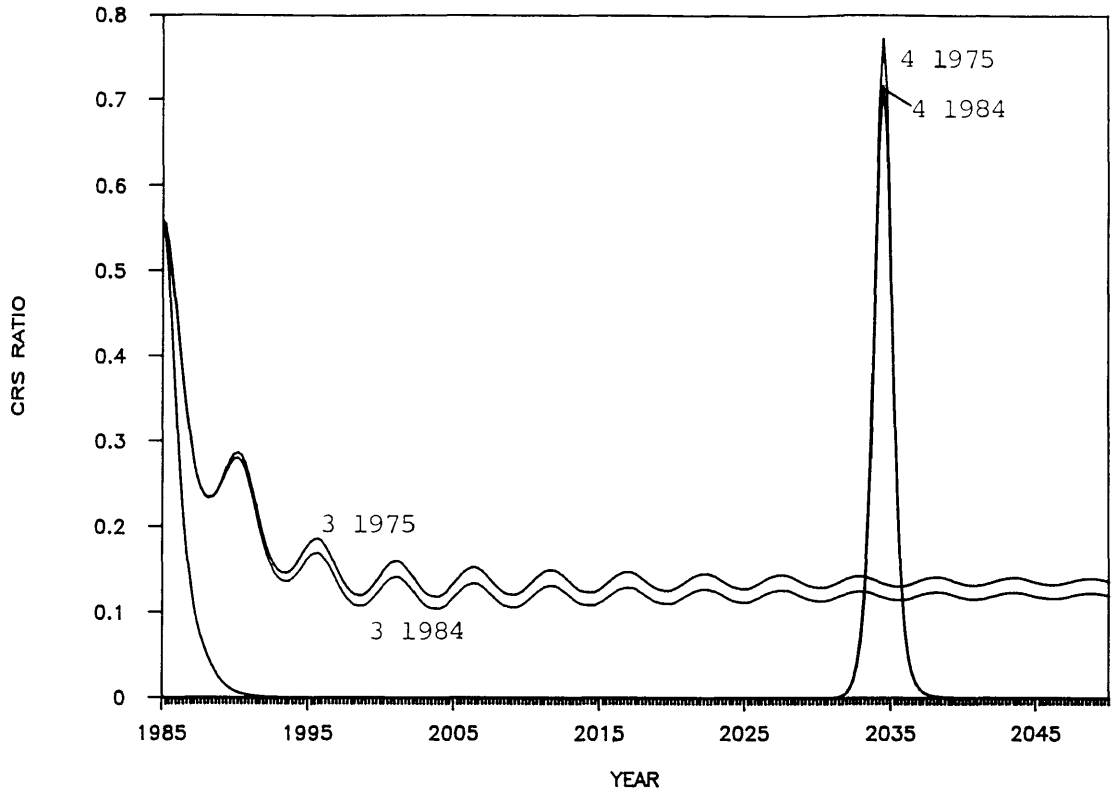
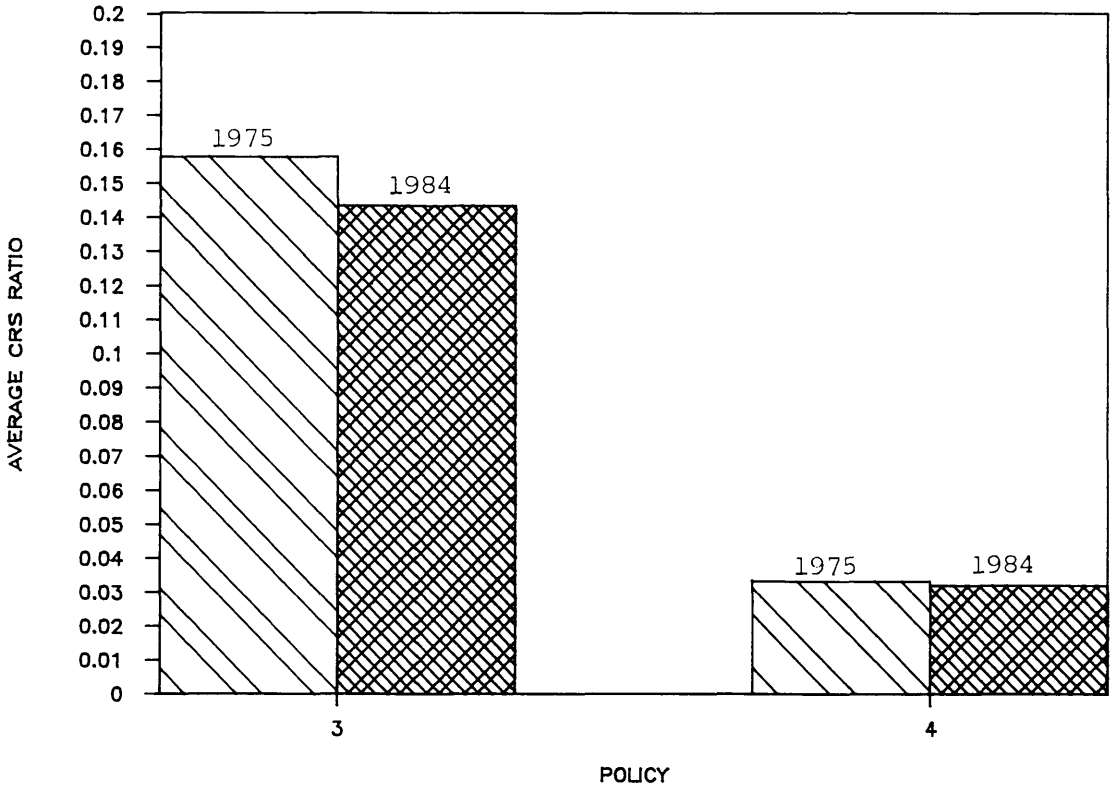

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
CRS RATIO
4 1975
4 1984
3 1975
3 1984
1985
1995
2005
2015
2025
2035
2045
YEAR

Fig.6.6


0.2
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
AVERAGE CRS RATIO
1975
1984
1975
1984
3
4
POLICY

policy of rubella vaccination. Fig. 6.5 illustrates the simulated impact on CRS incidence of the change in age-specific fertilty seen between 1975 and 1984 (Fig. 6.4) under a selective (policy 3) or a combined (policy 4) vaccination programme.

For both policies there is a decline in the CRS ratio as a result of the increase in average age at pregnancy. However, the reduction is less noticeable under the combined UK/USA policy. This is clearly seen in Fig. 6.6 which shows the long-term impact on CRS incidence (average CRS ratio between 1985 and 2050) of these changes in age-specific fertility, under each policy. These results may be explained by the effect of mass childhood vaccination which reduces the rate of transmission in the community, therefore offsetting the advantage of a longer period for females to become naturally immunized before pregnancy (created by the increase in age-specific fertility).

The current trend in age-specific fertility will probably be maintained with continued changes in attitude of women regarding the persuance of careers and delay in starting a family. That the benefits will be more pronounced under a selective style of policy may warrant consideration if the current immunization programme in this country is to be altered to a combined UK/USA policy in the future.

Throughout the remainder of the analyses 1984 age-specific fertility figures, as the most up to date data available, are used to describe the risk function, m(a) (see section 6.2(iii)), employed in calculating predictions

of CRS incidence (1985-2050).

(iii) The choice of vaccination strategy I

Temporal changes in the incidence of CRS (relative to the pre-vaccine era) under the influence of four different vaccination policies (characteristics of which are given in Table 6.2), are simulated in Fig. 6.7. The various changes to the single-stage selective policy begun in 1970 (i.e. policies 2-4), are implemented in 1985 and run until the year 2050.

The major point to emerge from Fig. 6.7 is the difference in the impact on CRS incidence between vaccination programmes in which childhood immunization is a component (policies 2 and 4) and programmes restricted to the selective vaccination of females (policies 1 and 3). The disparity in effects is a direct consequence of the relatively low force of infection in the late teenage and adult age classes (see Fig. 6.2a - estimates from S.E. England male serology), together with the tendency of mass vaccination to cause an upward shift in the age-distribution of the remaining susceptibles compared with that prevailing prior to immunization (such an effect does not arise for a selective policy where the majority of individuals are immunized via infection before the age of vaccination).

It is predicted that in the interepidemic periods, under a combined UK and USA policy, the incidence of acquired rubella may be so low as to result in virtual elimination of the infection (and, therefore, CRS), especially on a local

Fig.6.7

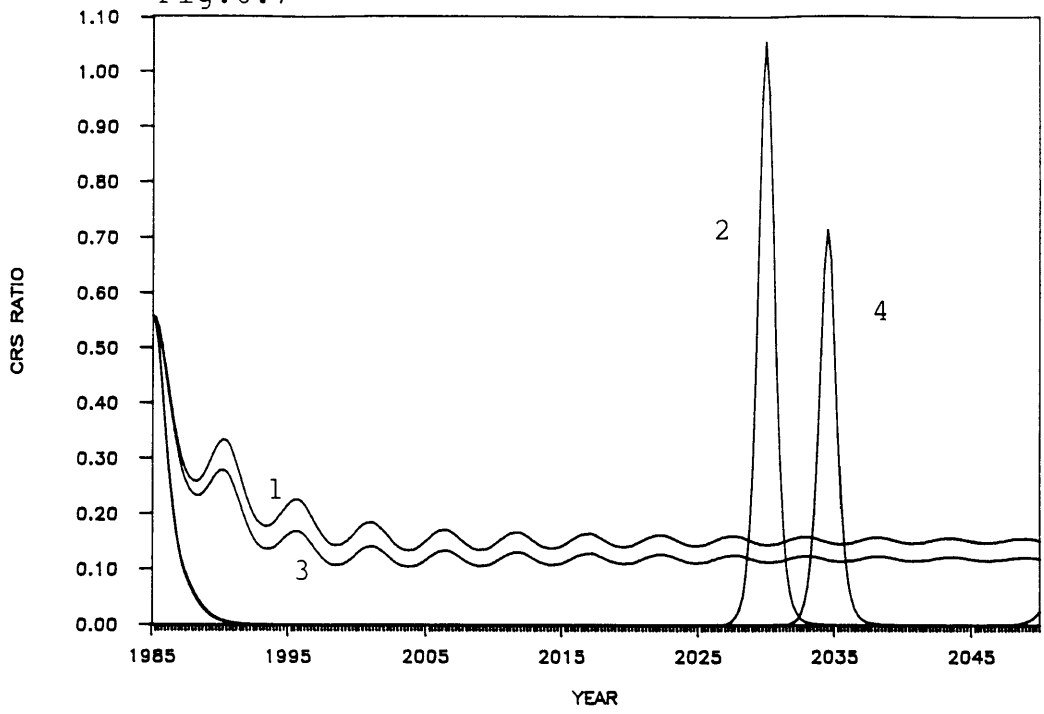


Fig.6.8

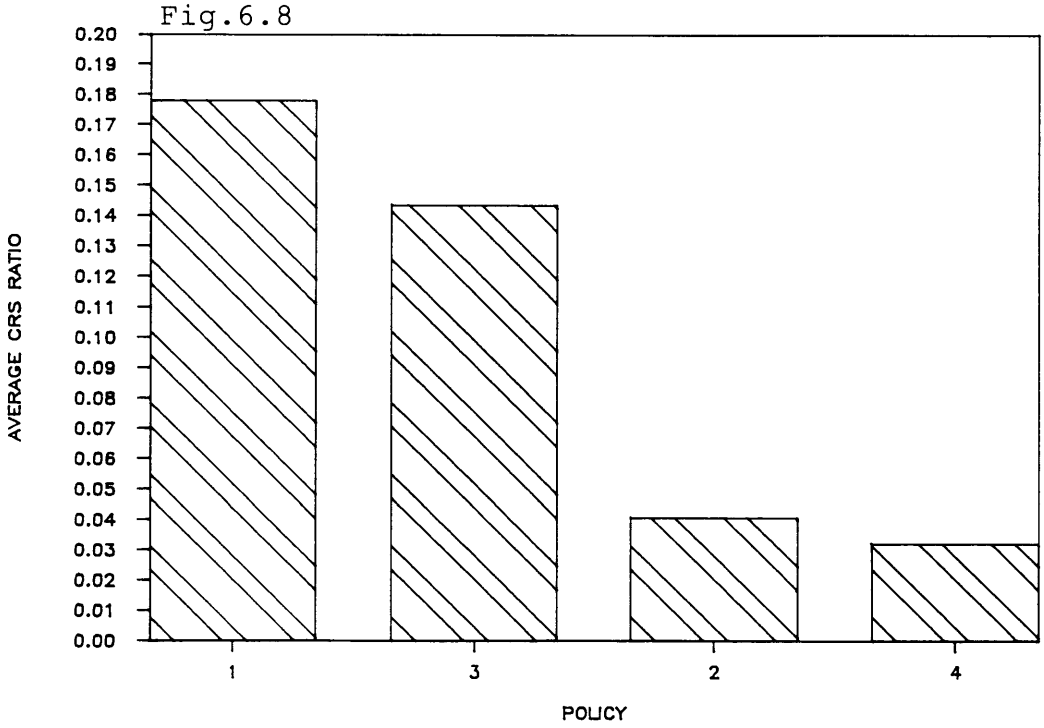


Fig.6.7 Choice of vaccination strategy I: temporal changes in CRS incidence. Predicted changes in the CRS ratio based on force of infection estimates derived from S.E. England male serology (see Fig. 6.2) are recorded for four different vaccination policies, labelled 1 , 2 , 3 & 4 (descriptions of which are given in Table 6.3). Other details are as for Fig. 6.3.

Fig.6.8 Choice of vaccination strategy I: long-term benefit. The average CRS ratio over the period 1985 to 2050 is recorded for each of the four vaccination policies simulated in Fig. 6.7. Labels are as defined in the legend to Fig. 6.7.

basis. However, as a result of spatial heterogeneity in vaccination coverage or the introduction of infection from other localities or from abroad, elimination of virus transmission is unlikely to be permanent. For instance, the average acceptance rate for measles vaccine in England in 1984 was 63%, but rates for the various Regional Health Authorities ranged from a maximum of 75% to a minimum of 52% (DHSS statsitics).

The consequence of spatial heterogeneity in immunity and infection is that outbreaks of rubella will be localised and they may not necessarily occur simultaneously across the country. Therefore, it will not be possible to predict accurately on a small local scale the time of CRS outbreaks (as might be inferred from Fig. 6.7), in order to take preventive action, such as promoting increased screening of adult women at risk in a defined locality.

A second observation from Fig. 6.7 is that immunization of adult seronegative women increases the effectiveness of both types of policy. Furthermore the magnitude of the peak in CRS under policy 4 is lowered, and by reducing the rate at which susceptibles are recruited following an epidemic, the period between CRS outbreaks is increased.

Despite the periodic outbreaks in CRS resulting from a combined UK/USA policy (policy 2 or 4), the overall reduction in CRS, shown as an average CRS ratio between the years 1985-2050 in Fig. 6.8, is predicted to be considerably greater than for a selective policy alone (policy 1 or 3). These results assume a level of vaccination of 2 year old boys and girls equal to the rate of measles vaccination in

this country at the present time (i.e. approximately 68%).

The effects of either a selective immunization programme (policy 3) or a combined UK/USA campaign (policy 4) on the prevailing levels of herd immunity to rubella in a community, are presented (for males and females separately) as ´serological surfaces´ in Fig. 6.9 & 6.10, respectively. Predicted age-serological profiles are shown quarterly for the years 1985 to 1999; this follows 15 years of single stage policy 1.

The substantive impact of teenage girl vaccination, and, later, the immunization of adult seronegative women, on the proportion of susceptible females of childbearing age may be ascertained by comparing Fig. 6.9a for females with Fig. 6.9b for males. The latter represents levels of pre-vaccination immunty in both sexes (males being little affected by selective immunization).

It is of significance in Fig. 6.9a that in 1985 (15 years after commencemennt of 10-15 year old girl immunization), the first cohort of vaccinees are in their mid-twenties, and not until the late 1990s do females throughout the age-span of childbearing years (16-40) derive protection from this policy (note the wave of elevated female vaccine induced immunity progressing from age 25 years to 40 years through the time period simulated). This supports conjecture in Chapter 3, Part 1, that further reductions in CRS incidence are to be expected under the current UK vaccination policy. Unfortunately, for the remaining small proportion of seronegative females between the ages of 16 and 40 years, the probability of acquiring

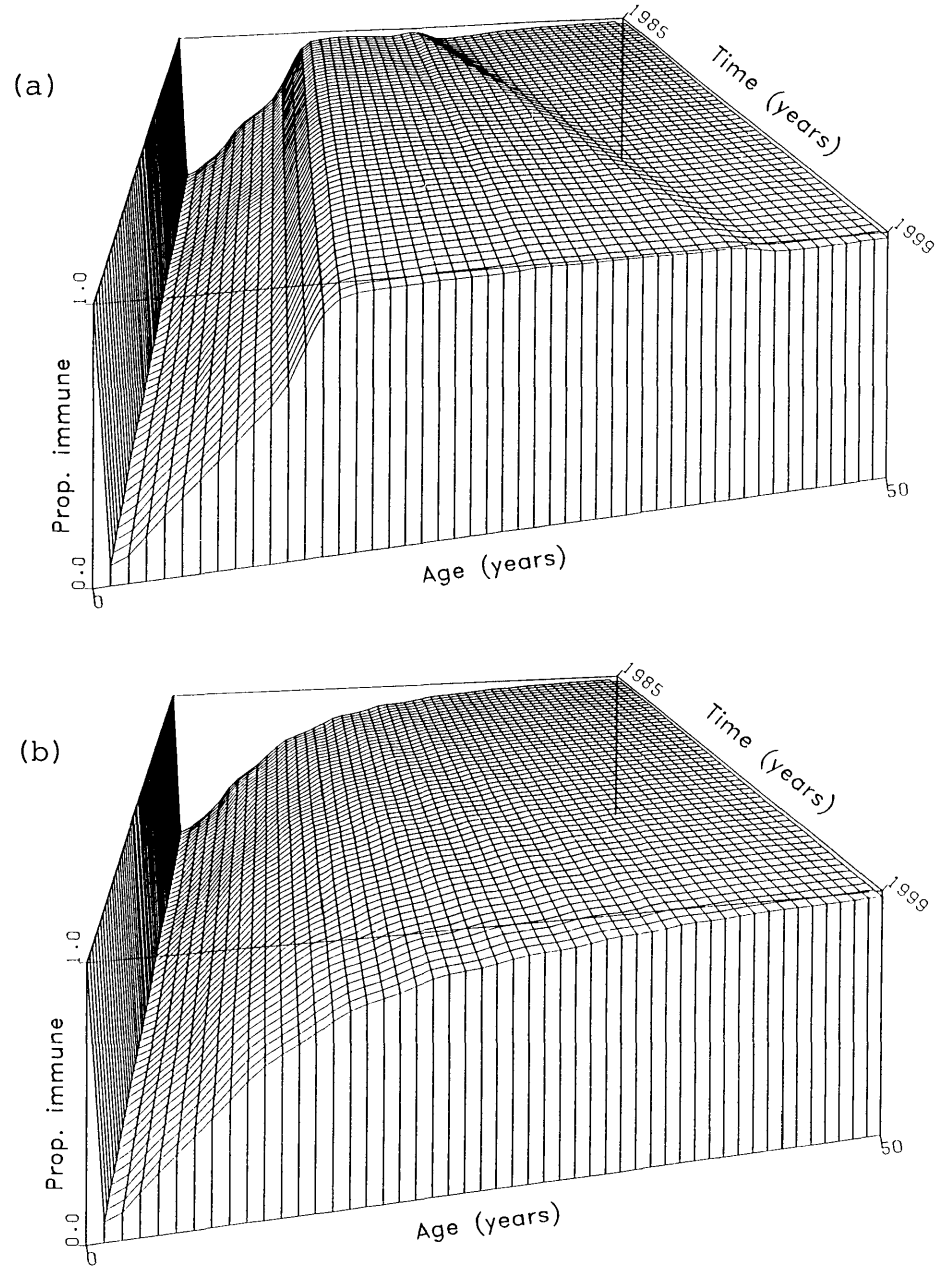


Fig.6.9 Choice of vaccination strategy I: policy 3 ´serological surface´. Time-dependent predictions of rubella ´age-serological profiles´ for females (surface a) or males (surface b), between 1985 and 1999, under the influence of a dual-stage selective immunization programme (policy 3 - Table 6.2). Immunity to infection may be derived maternally, from natural infection or from vaccination. Vaccination rates of c. 85% of 10-15 year old girls (1984 levels in Table 6.1) and 50% of 25 year old seronegative women are assumed, beginning 1985, following 15 years of policy 1. Predictions are based on age-dependent forces of infection estimated from S.E. England male serology.

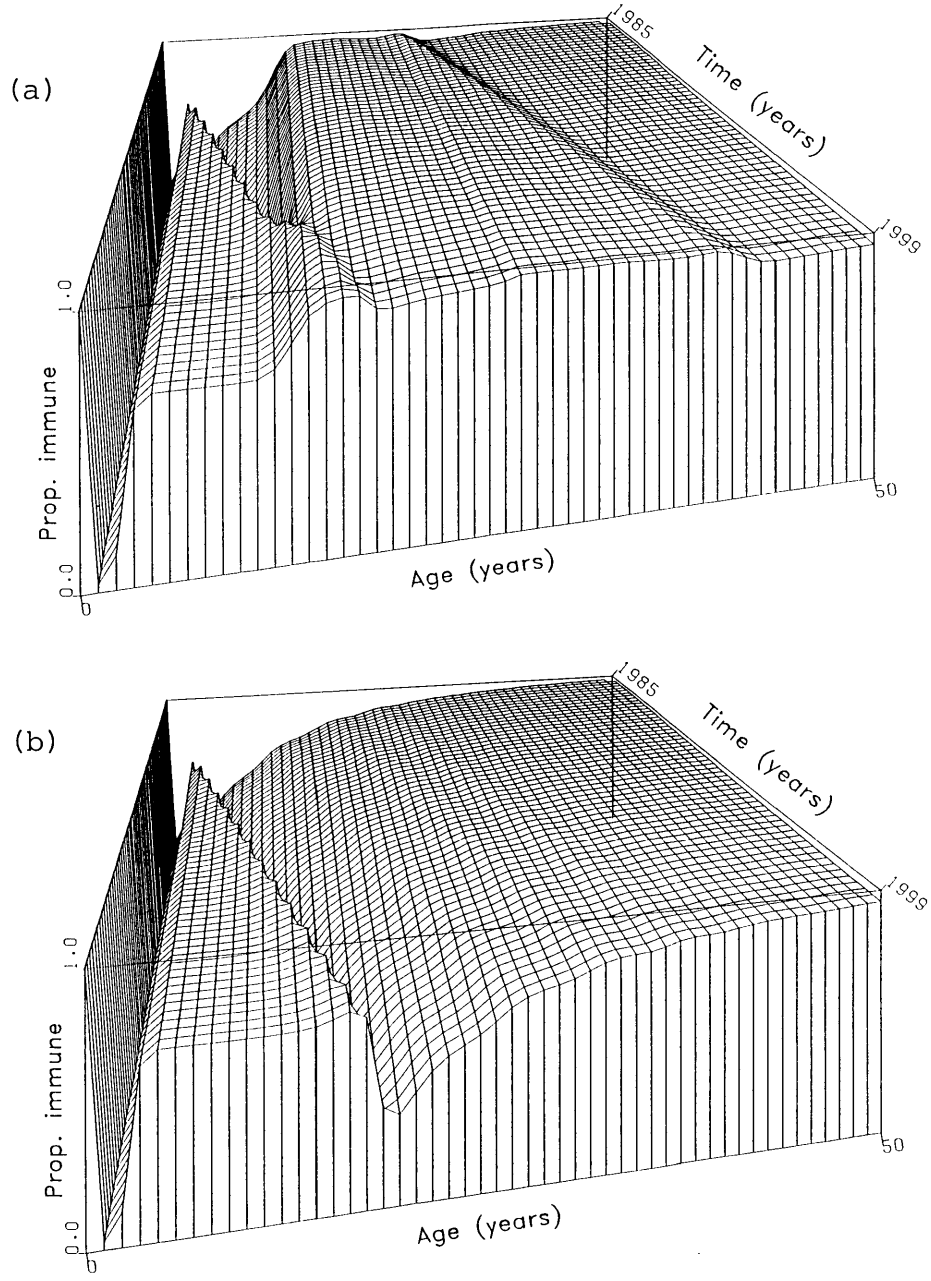


Fig.6.10 Choice of vaccination strategy I: policy 4 'serological surface'. The surfaces are the same as described in Fig. 6.9 except that changes in the proportion of individuals immune to rubella occur under the influence of a triple-stage combined UK and USA programme (see Table 6.2), in which the level of coverage of 2 year old boys and girls is assumed to be 68% (the proportion vaccinated against measles in England in 1985).

rubella during pregnancy is unaltered by policy 3. It is for this reason that only 100% coverage of women in the 'at-risk' years will be adequate to eliminate CRS under policies 1 and 3.

Under a mixed UK and USA immunization programme, the situation is rather different. Elevated levels of childhood immunity resulting from vaccination of 2 year old boys and girls (Fig. 6.10a & b), restricts the number of infectious individuals in the community, thus reducing the probability of susceptibles (who escape vaccination) acqiring rubella immunity naturally. As a consequence the prevailing levels of herd immunity in teenagers and adults under this combined policy may actually be lower than for selective immunization alone (compare Fig. 6.9 with Fig. 6.10). The buildup of susceptibles in the older age classes is potentially dangerous. It may lead to an outbreak of rubella after many years of childhood immunization (and subsequently CRS) as seen in Fig. 6.7. The policy of vaccinating all adult women found to be seronegative reduces the pool of female susceptibles between the ages of 16 and 40 years and thereby limits the scale of the long term periodic outbreaks of CRS.

(iv) Trends in vaccine uptake

Much of the controversy over which policy of rubella immunization should be adopted in the United Kingdom in the future, may be attributed to disagreement over achievable vaccination rates, now, and in years to come. Theoretical

studies suggest that unless mass vaccination covers a large proportion (in excess of 60%) of each cohort soon after birth, no substantial advantage is to be gained by a change from the current policy (Anderson and Grenfell, 1986). It should also be noted that vaccination rates will vary considerably over the country (mentioned in the previous subsection), such that in some areas the level of coverage may well be considerably less than the national average.

Some inferences of significance to resolving this problem may be derived from observations of the trend, over recent years (1976 to 1985), in the vaccination coverage for rubella and measles (Fig. 6.11) in England (data come from DHSS Statistical Division, SBL 607, 1985). It seems that the rate of rubella immunization of 10-15 year old girls has reached a ceiling at around 85%. The full effect of this high rate of coverage on CRS incidence is, as yet, not fully evident (see section 3.3), nevertheless, unless immunization of adult seronegative women can fully account for the 15% deficit, CRS will continue in this country.

In contrast, measles vaccine coverage has been steadily rising in the 1980s (Fig. 6.11) and a continuation of this trend seems probable (as long as serious vaccine side effects, which will almost inevitably occur in the future, are kept in perspective by the media and the general public). If it is assumed that mass vaccination of boys and girls for rubella would match the rate observed for measles (a reasonable supposition were the two vaccines administered together), then there is every indication that high rates of childhood rubella vaccination, as part of a combined UK/USA

Fig.6.11

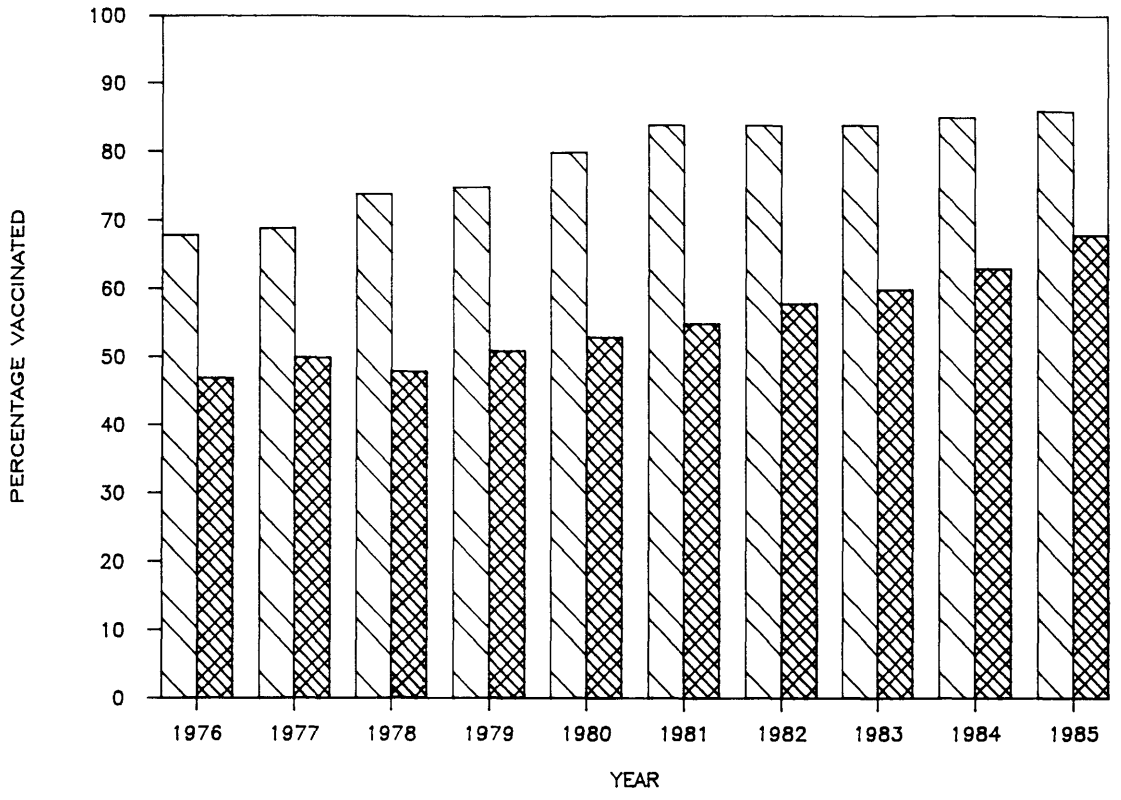


Fig.6.11 Trends in vaccine uptake: rubella and measles 1976-85. The annual percentage of schoolgirls vaccinated by the age of 15 years against rubella (▨) and of boys and girls (by the end of their second year) vaccinated against measles (▩) are recorded for the years 1976 to 1985. Data were provided by DHSS statistical division.

Fig.6.12 Trends in vaccinae uptake: temporal changes in CRS incidence. The impact of a selective vaccination programme, policy 3 (labelled 0%), and of a combined UK/USA programme (policy 4) for various levels of vaccination of 2 year old boys and girls (60, 68, 75 and 80% - as labelled, except 75 = 80%) on the CRS ratio, are compared. Other details on vaccination and transmission rate estimates are as for Fig. 6.7.

Fig.6.13 Trends in vaccine uptake: long-term benefit. The average CRS ratio between the years 1985 and 2050 has been calculated from the simulations shown in Fig. 6.12. Labels for the histogram are the same as in Fig. 6.12.

Fig.6.12

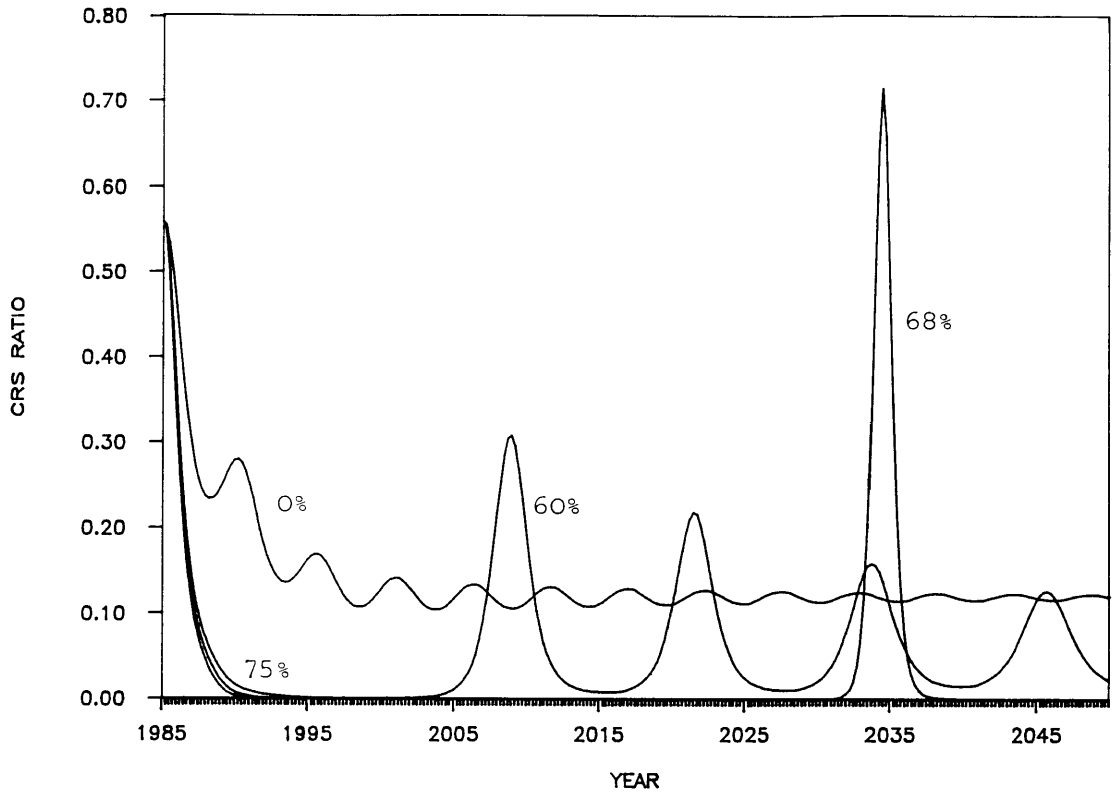
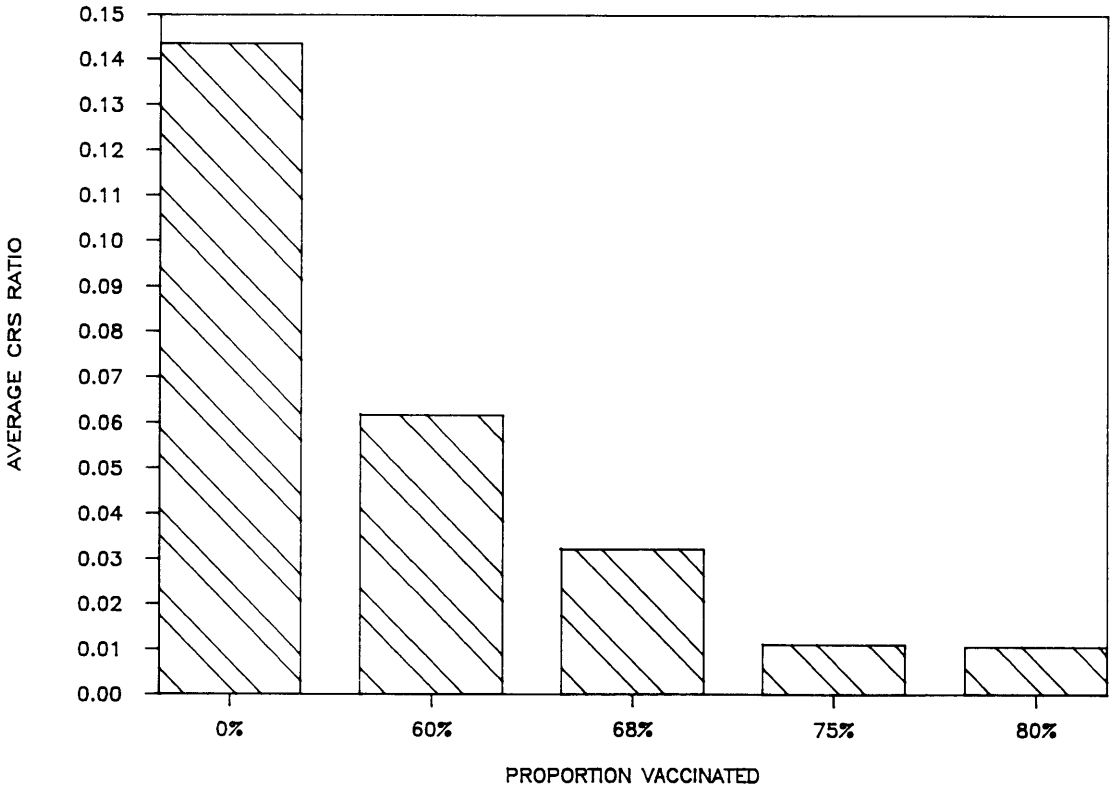


Fig.6.13



policy, would be achieved at the present time (measles rate currently at 68%), with likely increments in uptake in the coming years.

Quantitative predictions to substantiate the preceding conjecture are shown in Fig. 6.12 & 6.13. Predicted temporal changes in CRS ratio under a dual stage selective vaccination policy (policy 3) or under a triple-stage combined UK and USA programme (policy 4) for various levels of vaccination of 2 year old boys and girls (60, 68, 75 & 80%) are presented in Fig. 6.12. As the level of mass vaccination of children increases so its impact on CRS incidence becomes greater. This is reflected in Fig. 6.13 which shows a progressive improvement in the long-term benefit derived from policy 4 with each rise in vaccination coverage, over that obtained from the selective policy 3 (for which the proportion vaccinated is assumed to remain stable at about 85%).

As a result of these increases in childhood vaccination coverage, the rate of recruitment of susceptibles following an epidemic is reduced, and the number of susceptibles required to support an epidemic increases (because (a) the effective reproductive rate of the infection is progressively lowered as a result of fewer infectious individuals in the community, and (b) more of the remaining susceptibles are shifted into older age classes in which there is a lower probability of being infected).

One consequence of these effects is the increasing interepidemic period shown in Fig. 6.12, seen between the predictions for 0% (i.e. policy 3), 60 and 68% (policy 4)

coverage of children. At 75% or above, the period between epidemics is so long that CRS is essentially eliminated. A second, less desirable, consequence of these rises in vaccination rate, is an increasing magnitude of the epidemics (because of the rise in the average age of susceptibles and the need for more susceptibles to spark off an epidemic).

Even though the long-term benefit of policy 4 is considerable (Fig. 6.13), the occurrence of large outbreaks of CRS, emphasised by intervening periods of complete or near complete absence of the disease, will be of great public concern and maybe looked upon as a failure of the vaccination strategy. This is clearly not the case, the long term large scale oscillations are a consequence of the dynamic changes in virus transmission induced by high vaccination coverage.

(v) The choice of vaccination strategy II

Having adopted a combined selective and mass vaccination programme, such as policy 4, it would seem good sense to continue with all three stages until the first cohort of two year old vaccinees has attained the age of 15 years (so covering the gap between childhood and teenage immunization), and then, as a cost-effective measure, to drop the two selective stages of the policy therafter.

The serological surfaces shown in Fig. 6.14 & 6.15 show what is predicted to happen to female and male levels of herd immunity between the years 2000 and 2015, following 15

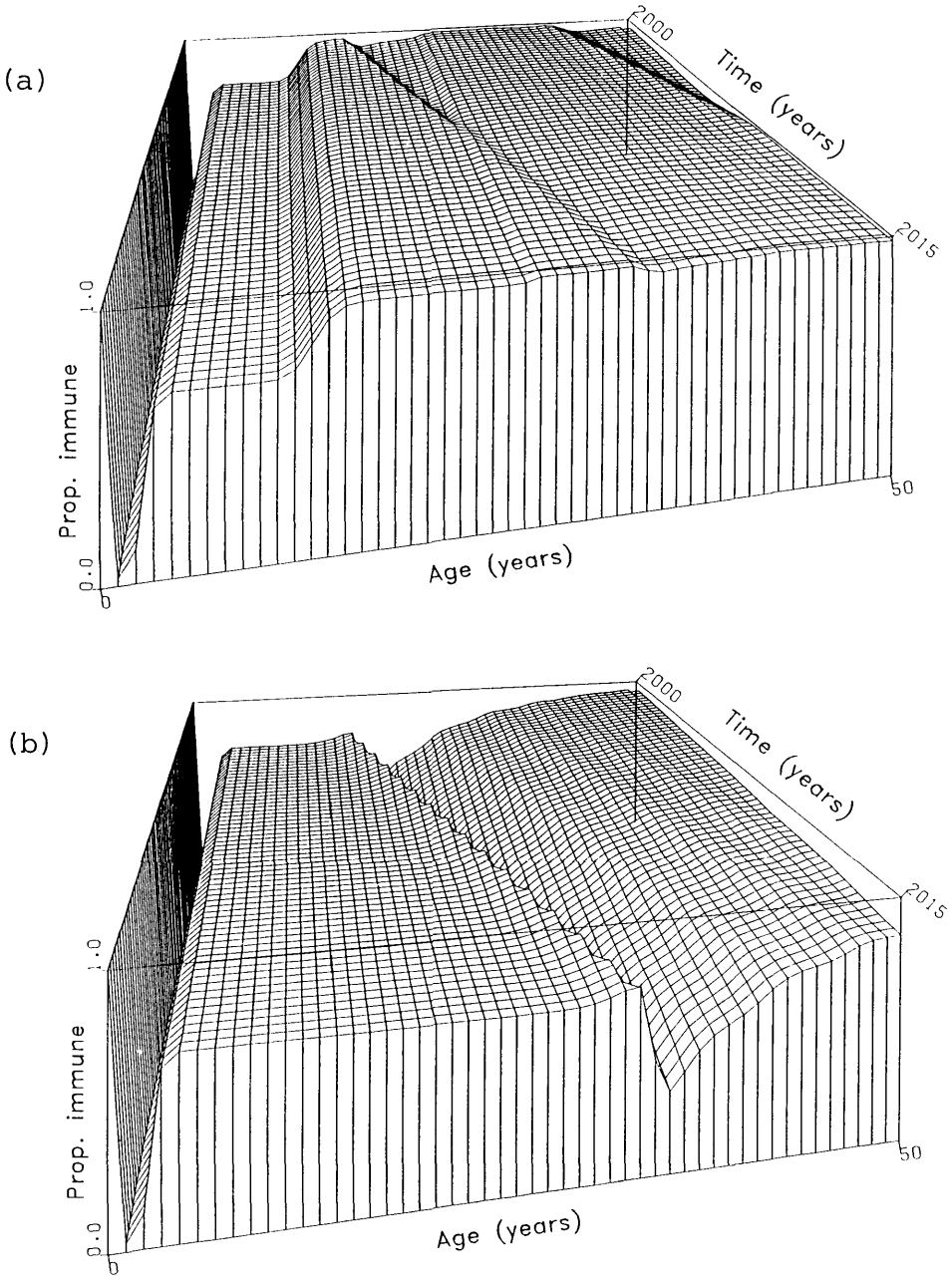


Fig.6.14 Choice of vaccination strategy II: policy 4 ´serological surface´. Age- and time-dependent predictions of the change in the proportion of individuals in the community immune to rubella infection, under the influence of a triple-stage combined vaccination programme (policy 4). The surfaces for females (a) and males (b) cover the time period 2000-2015 and follow on directly from those shown in Fig. 6.10.

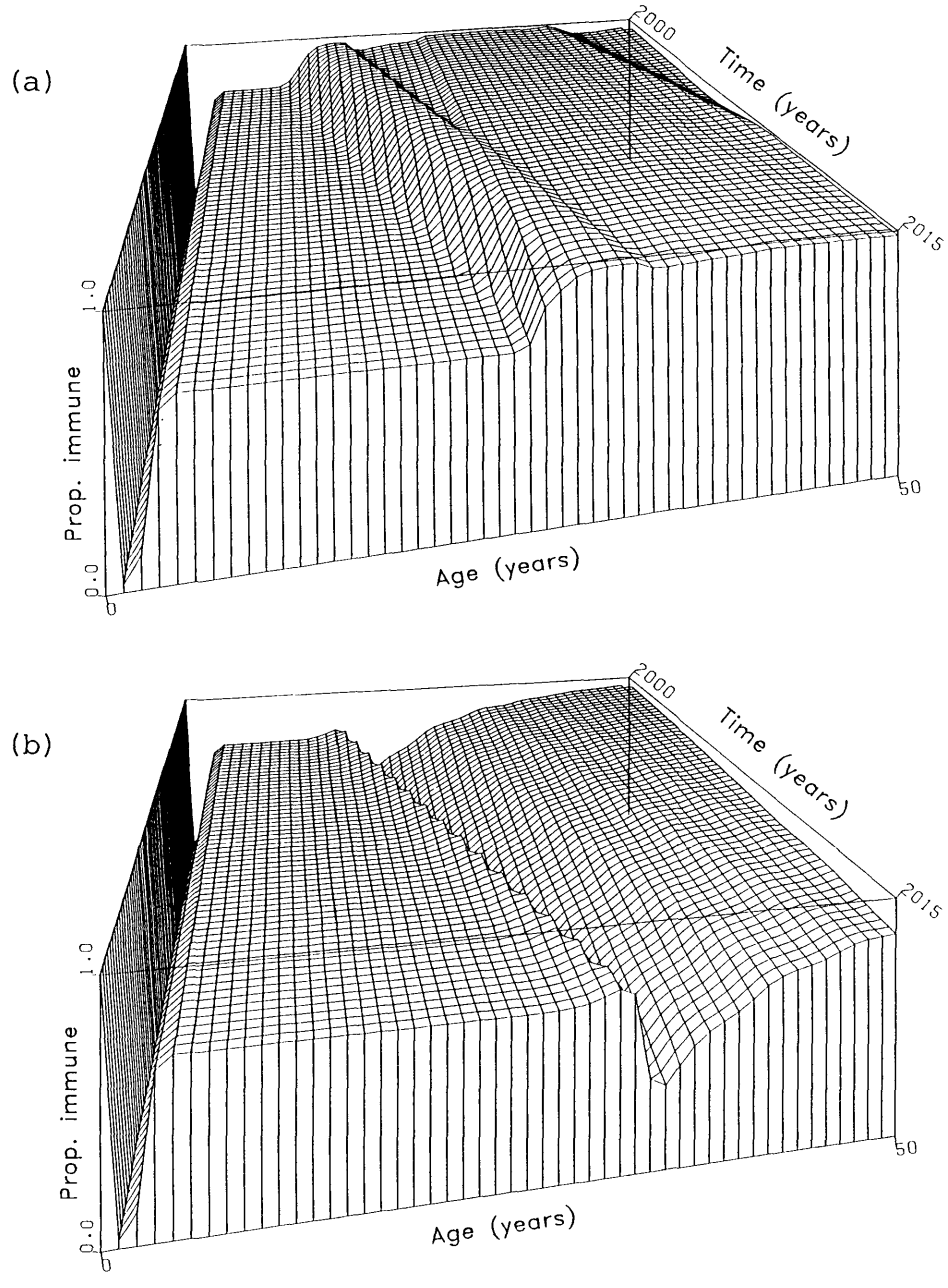


Fig.6.15 Choice of vacciantion strategy II: policy 4 ´serological surface´ without selective immunization. Similar to Fig. 6.14 except that the selective stages of female vaccination are dropped in the year 2000 for the duration of the simulations, leaving only immunization of 2 year old boys and girls.

years of policy 4 (shown in Fig. 6.10), under the conditions of either a continuation of all three stages of the programme (Fig. 6.14), or cutting out selective immunization, leaving only childhood vaccination (Fig. 6.15).

As a consequence of mass childhood immunization the probability of being infected with rubella is reduced for susceptibles of all ages and both sexes. The effect of this is seen in each serogical surface (Fig. 6.14 & 6.15). After immunization of 2 year olds (set at 68%), there is little rise in levels of herd immunity as a result of natural infection. Only following selective immunization of 10-15 year old females (and, later, 25 year old seronegative women) is this 'immunity gap' reduced (Fig. 6.14a). However, without these selective stages, the level of immunity gained from 2 year old vaccination is not substantially built upon, with increasing age, and the deficit in herd immunity progressively encroaches into the childbearing years (Fig. 6.15a) creating a significant pool of seronegative women of childbearing age.

The consequence of large numbers of susceptible women in the 'at-risk' years to the incidence of CRS is shown in Fig. 6.16a & b for various levels of childhood vaccination (60-85%). The inevitable result is a series of epidemics of rubella infection and, in turn, large peaks in CRS incidence. Increasing the vaccination coverage lengthens the interepidemic period, but also increases the magnitude of the outbreaks when they do occur (for the reasons previously stated), until coverage is high enough to prevent

Fig.6.16

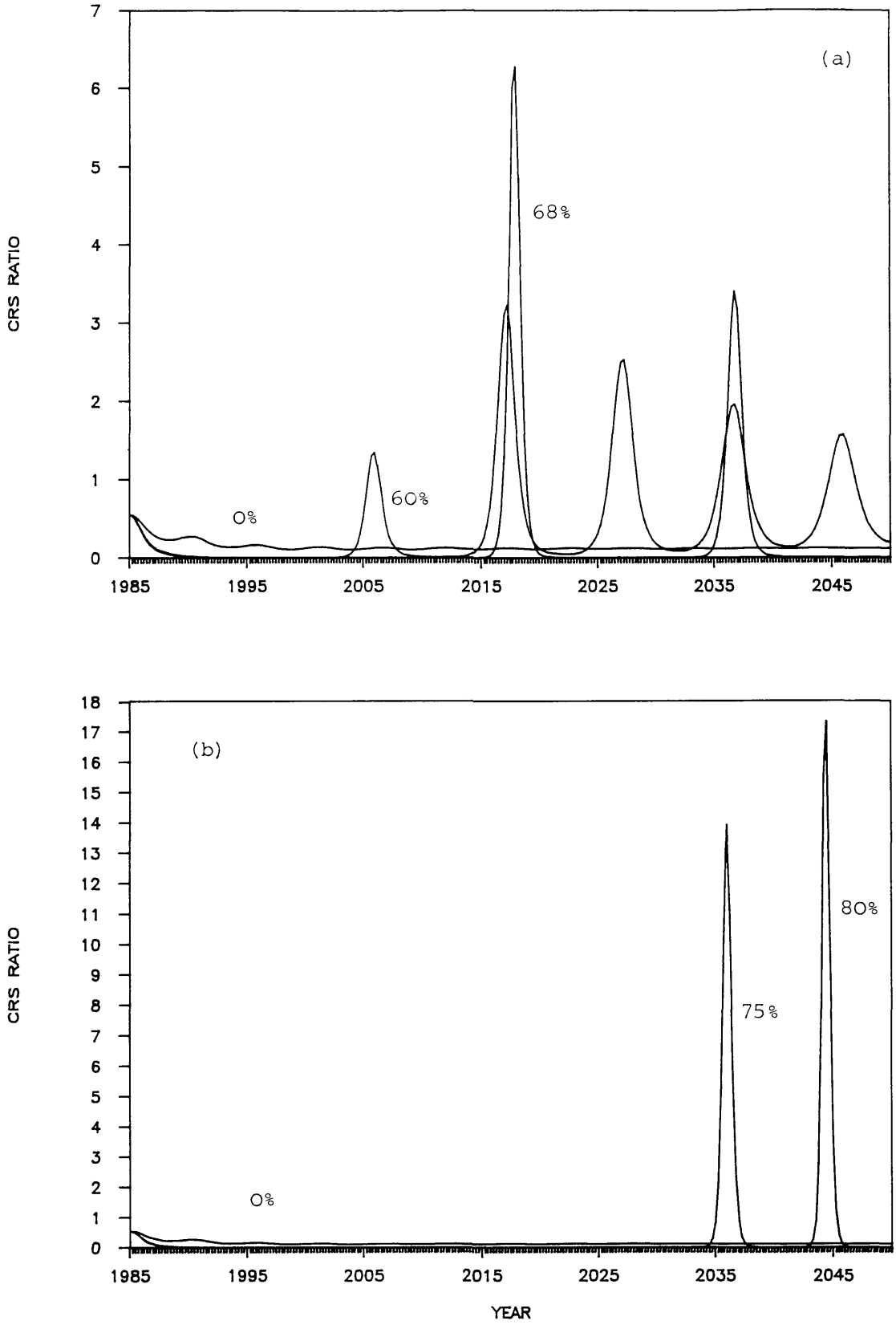

(a)
CRS RATIO
0%
60%
68%
(b)
CRS RATIO
0%
75%
80%
1985
1995
2005
2015
2025
2035
2045
YEAR

Fig.6.17

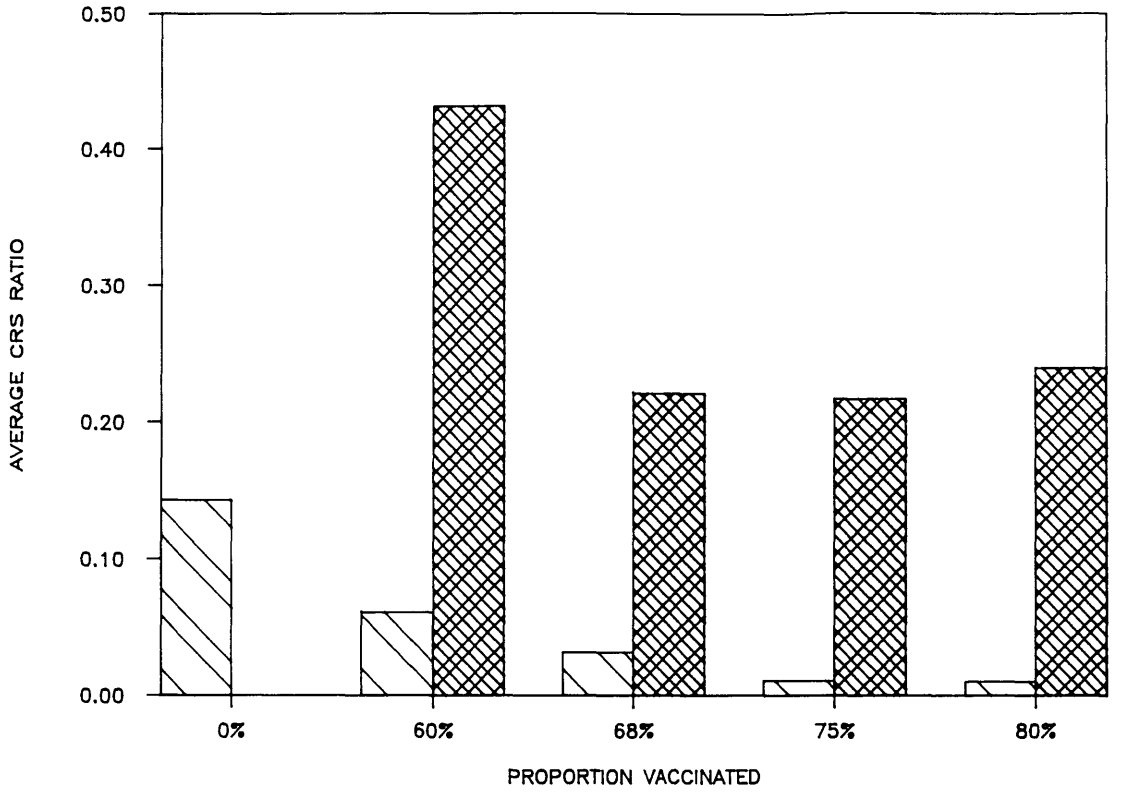


Fig.6.16 Choice of vaccination strategy II: temporal changes in CRS incidence. The predicted changes in CRS ratio between 1985 and 2050 under the impact of policy 4 with various levels of mass childhood vaccination (0, 60 and 68% in graph a; 0, 75 and 80% in graph b). The selective stages of the immunization programme are cut out from 2000 onwards (following 15 years of a combined UK/USA programme - policy 4) for all rates of immunization except 0%. In other respects the graph is similar to that shown in Fig. 6.12. The simulation with 0% childhood immunization is equivalent to policy 3 (Table 6.2) and is shown for comparison. At 85% CRS is eliminated.

Fig.6.17 Choice of vaccination strategy II: long-term benefit. Average CRS ratios over the time interval 1985 to 2050 for increasing levels of childhood immunization, as part of a triple-stage vaccination programme (policy 4). The histogram compares the benefit derived from continuation of all three stages throughout the time period, ▨ (taken from Fig. 6.13), with that obtained if the selective stages are dicontinued after 1999, ▩ (calculated from the simulations shown in Fig. 6.16). The average CRS ratio for policy 3 (0%) is included for comparison.

perpetuation of the virus in the community (> 80%).

The long-term benefit of policy 4 (see Fig. 6.17) will, therefore, be substantially impaired by cutting out the selective stages of the strategy, at all levels of coverage less than that required for eradication of rubella (between 80 and 85%), even when compared with the continuation of a dual stage selective campaign alone (0%, i.e. policy 3).

6.4 Discussion

The strongest impression created by the foregoing studies is the complexity of the interplay between community vaccination and the transmission dynamics of an infectious agent. It is clear that the introduction of a rubella immunization programme into a community may have undesirable consequences to the incidence of postnatal and congenital rubella, and to herd immunity levels, which are not intuitively forseeable. There is obvious advantage to be gained from predicting such consequences prior to implementing a particular vaccination policy, but this would be difficult without the aid of a mathematical model.

If predictions based on theory are to be of use to those making decisions on vaccination strategy, then there must be assurance that the model accurately represents the epidemiology of the infection. This requires not only that it is based upon valid assumptions about the infectious disease, but that parameter estimates are derived from reliable empirical information (Fine & Clarkson, 1983).

Refinements in the model employed in this investigation (see Anderson & Grenfell, 1986; Anderson & May, 1985a) which take account of information on heterogeneity in transmission between age groups and in 'who acquires infection from whom', represent important steps in satisfying these requirements.

Unfortunately there is still concern over the accuracy of estimated values of age-related forces of infection. Results from the present study reiterate those of Anderson and Grenfell (1986) in showing a marked dependency of the predictions on the nature of change, with age, in transmission rates, particularly on what decline occurs between the schoolage classes (5-15 years) and adult age classes (20+ years). In Chapter 4 serious doubts concerning the accuracy of case notification data for the provision of estimates of transmission rates were raised. There are also problems over the reliability of serological information in relation to (i) the mode of collection (Chapter 3, Part 1), (ii) the loss of detectable antibody many years after infection (Chapter 5, Part 1), and (iii) genetic association with antibody production and maintenance (Chapter 5, Part 2).

However, the impact of these possible sources of error on the accuracy of $\lambda(a)$ estimates based on serology used in this study is thought to be minimal. Significant bias in the collection of samples for the surveys of S. Yorkshire and S.E. England has already been argued against in section 3.2. False seronegatives arising from genetic non-susceptibility or decay in antibody potency are unlikely to

cause considerable changes to the proportion immune where the susceptible proportion is relatively large. This includes all age classes up to and including the childbearing ages (1-40 years), for which (a) values have been determined (see Table 3.2). Nevertheless, further research is necessary to firmly establish the importance of these factors in this respect.

Model predictions based on serologically estimated infection rates are therefore regarded as more reliable than those based on case notifications. Of the two sets of parameters based on serology, those determined for males from the S.E. England survey were thought to most accurately represent pre-vaccination rates of infection essentially on the basis of sample size (see Part 2 of Chapter 3). However, it is important to note that the general age-dependent trend in the force of infection for S. Yorkshire was broadly similar to that for S. E. England.

Under the assumption that the age-dependent changes in the force of infection estimated from the male serology from S.E. England are reliable, and the level of uptake of rubella vaccine by 2 year old males and females would mirror that for measles (currently 68%), the model predictions suggest that significant benefit would be gained by adopting a mixed UK/USA strategy in preference to the current policy. Further support for such a decision comes from the recent trend for increase in measles vaccine uptake, and the speculation that a double vaccine, i.e. measles and rubella would be a stimulus to vaccine acceptance (two for the price of one, see Report, 1986).

However, there still remain some doubts concerning the implementation of a dual- or triple-stage policy. As was highlighted by the crisis in pertussis vaccination in the 1970's (Fine & Clarkson, 1984; Anderson & May, 1982a) the way the public perceive disease as a result of man-made, as opposed to natural, causes when a policy has been successful in reducing the incidence of infection to very low levels can have serious consequences on vaccine acceptance (Anderson & May, 1982b). In a similar way the possible long-term recurrent epidemics of CRS predicted to arise as a result of changes in the infection dynamics induced by mass vaccination, may be misconstrued (by the public and the medical profession) as a failure of the policy, leading to reductions in the rate of vaccine uptake. The serious adverse effects of low level childhood immunization on the incidence of congenital rubella need not be restated.

Thus for reasons of safety there is a case for maintaining the current selective vaccination policy (which has little effect on the transmission dynamics of rubella virus) until (i) further rises in the proportion of children vaccinated against measles gives adequate assurance that high levels of childhood vaccination could be maintained for rubella in the future, and (ii) there is greater confidence in the accuracy of empirical data.

This hesitation should not be regarded as being unnecessarily cautious because poor judgement now could have adverse consequences on CRS incidence in the future. Furthermore, it should not be forgotten that the current policy is proving very successful and reductions in the

incidence of CRS look like continuing in the future.

A final point refers to the economics of establishing a multi-stage rubella vaccination policy. The introduction of mass childhood immunization against rubella, even if administered in conjunction with measles vaccine will be an expensive outlay. However, the predictions of the model suggest that unless childhood vaccination levels are in excess of 80%, then all stages of the policy will have to be maintained indefinitely. The impact of cutting out selective immunization some time in the future on grounds of economy would leave a large susceptible fraction in the childbearing years (as a result of changes in herd immunity induced by vaccinating children) which could have disasterous consequences on CRS incidence.

SUMMARY AND CONCLUSIONS

1) The aim of the research presented in this thesis was to accumulate detailed seroepidemiological information for rubella in England with which to (a) gain a better understanding of the transmission dynamics of this infection in the community, (b) assess the impact of the current selective immunization programme and (c) satisfy the need for accurate empirical data in mathematical models used to evaluate the merits of possible future rubella vaccination policies.

2) The epidemiological studies were based, primarily, on finely age-stratified collections of serum samples (comprising over 6000 specimens) from South East England between 1980 and 1984, and from South Yorkshire for 1969 and a number of years since the onset of rubella immunization in this country. These surveys adequately represent horizontal cross-sectional samples from the two study areas although future surveys would be improved by prospective random sampling of better defined sections of the population.

3) The radial haemolysis test, used to screen the serum samples for rubella-specific antibodies, was well suited to serological surveillance, proving simple to perform, rapid,

economical and sensitive to low level antibody (2.5-3.5 iu/ml). Simple modifications to the basic test procedure provided quantitative estimates of rubella antibody in sera during routine screening.

4) A number of interesting observations have been made from age-serological profiles (ASPs) of the two study areas:

(i) Changes, with age, in the proportions of males and females serologically positive for rubella (similar for both S.E. England and S. Yorkshire) are typical of endemic childhood viral infections, with approximately 90% of the population possessing specific antibody by the age of 20-24 years;

(ii) Overall levels of seropositivity remain largely unaltered since the initiation of immunization and evidence for indirect effects of female vaccination on male immunity levels is insubstantial. Such features are to be expected under a selective immunization campaign;

(iii) However, a significant decline in the proportion of seronegative women of childbearing age as a result of schoolgirl immunization is, today, evident in both survey populations;

(iv) The raised levels of seropositivity do not extend throughout the 'at-risk' years of fertility suggesting that in some parts of the country immunization of adult

seronegative women is not having an appreciable impact and, also, that the full effect of adolescent female vaccination will not be manifest until the late 1990´s;

(v) A small proportion of the population remain serologically negative for rubella in the upper age (greater than 60 years) classes. Resolving the factors underlying this result is thought to be of importance in establishing the validity of information on rubella virus transmission derived from age-serological profiles.

5) Formal analysis of the age-stratified serological data from S.E. England for males (basically unaffected by immunization) and from S. Yorkshire 1969 (i.e. pre-vaccination) using a ´polynomial catalytic infection model´ revealed a marked age dependence in the rate or force of rubella infection. The per capita rate of transmission was shown to vary from low in young children to high in older children and early teenagers (5-15 years of age) and back to low again in adults. Schooltime interaction is thought to be the major generating factor.

6) The mean age at rubella infection was calculated to be 10.8 years for S.E. England (male data) and 9.4 years for S. Yorkshire 1969. On the basis of sample sizes most confidence rests with the estimate from male serology.

7) Estimates of the force of infection determined from scant rubella case reports were, in general, less dependent upon

age than those derived from the serology. Doubts were raised regarding the accuracy of epidemiological information derived from case notifications due to (a) age-dependent bias in reporting and (b) an assumption that all individuals are infected with rubella during their lifetime; a supposition not supported by observations from ASPs.

8) Analysis of quantitative rubella antibody measurements revealed a significant decay in mean antibody concentrations with increasing age (consistent for all survey years), and a concomitant increase in antibody concentration variability. This suggests that serological analysis may not always provide evidence of long-past infection, a phenomenon which could have some bearing on the shape of ASPs from which estimates of transmission rates are determined.

9) Significantly lower level antibody was observed in female age groups influenced by vaccination when compared with males of the same age. Compounded by declining antibody concentrations with age, and immunity which is less solid than that naturally induced, this may lead to problems in a population for which herd immunity is maintained by vaccination, as with a mass childhood immunization policy.

10) Additional collections of samples from HLA and ABO typed individuals were obtained for an investigation of the role of genetic factors in determining rubella antibody concentrations. Analysis of unrelated juvenile chronic arthritis patients uncovered strong associations between

(a) HLA-DR5 and high level antibody (which was most probably linked to vaccine-induced antibody) and (b) HLA-DR4 and seronegativity. There was only tentative, indirect confirmation of these associations from samples of normal, healthy individuals, and the results of sibling analysis in two family studies were negative. The importance of inherited chracteristics to rubella antibody levels in the general population remains inconclusive and this issue requires further investigation.

11) A number of alternative rubella vaccination policies for the UK have been examined using an advanced mathematical model of rubella virus transmission. Predictions of the impact of mass childhood immunization programmes on the incidence of CRS were found to be highly dependent upon the pattern of age-related changes in the force of infection (in particular the differences seen between the serological and case notification estimates). This observation highlights the importance of accounting for known epidemiological characteristics in model structure whilst emphasising the need for precise estimates of parameters.

12) The accuracy of serological estimates of these model parameters has been considered in relation to (a) age-related trends in mean antibody concentrations, (b) genetic non-susceptibility and (c) sampling bias in the surveys. Such factors were thought to be of minor significance (in this project) compared with the gross inaccuracies of the alternative source of empirical data,

i.e. case reports. Nevertheless, additional research in this area is needed.

12) The main conclusions drawn from the model simulations of CRS incidence are as follows:

(i) Assuming rubella vaccine uptake by children matches that for measles (currently 68%) and the present trend for increase in measles immunization continues, significant benefit would be gained by changing to a combined UK and USA strategy (i.e. selective and childhood immunization) in the future;

(ii) As a result of herd immunity effects of mass cohort immunization and a low rate of infection in the childbearing age classes a combined policy might give rise to long-term periodic outbreaks of rubella and subsequently congenital rubella which should not be looked upon as a failure of the policy;

(iii) The predicted decline in herd immunity levels resulting from the interruption of rubella transmission in the community dictates that for levels of immunization less than 85% selective as well as childhood vaccination must continue indefinitely under a combined policy;

(iv) Immunization of adult susceptible women forms a significant part of the control measures against congenital rubella. However, the absence of national estimates of the

proportion of this segment of the population who have recieved vaccine prevents any quantitative predictions of its role in current and future vaccination policies;

(v) Given the uncertainty over accuracy of parameter estimates and over future levels of rubella vaccine uptake, there remains a strong case for continuation of the current selective policy in the UK, until such problems are resolved.

13) The analyses used for this study of rubella are suitable for other childhood viral and bacterial infections providing that they are directly transmitted and produce long-lasting immunity to re-infection. Consequently future work in this area should concentrate on (a) monitoring the effects of other immunization campaigns in progress in this country, such as for measles and pertussis and (b) evaluating the need and the optimal strategy for the control of common vaccine-preventable infections like mumps and chickenpox.

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APPENDIX 1

Equipment required for the radial haemolysis methods described in Chapter 2

(1) Autoclave or microwave oven.

(2) Waterbath(s) with variable temperature control and circulation mechanism.

(3) Microhaematocrit, packed cell volume reader and capillary tubes.

(4) Centrifuge taking 30 ml screw capped, flat bottomed, glass universals.

(5) Square gel plates. Method I: Sterilin 10x10 cm petri dishes (Sterilin Ltd., Sterilin House, Clockhouse Lane, Feltham, Mddx TW14 8QS);

Method II: Nunc 23x23 cm bioassay plates (Gibco Ltd., P.O. Box 35, 3 Washington Road, Paisley, Scotland PA3 4EF).

(6) Levelled surface adjacent to water bath.

(7) Steel tubular borer 3 mm diameter (Shandon Southern Products Ltd., Chadwick Road, Astmoor, Runcorn, Cheshire, WA7 1PR) connected to a vaccuum line and trap.

(8) Graduated eyepiece lens x10 mag. (Jencons MM-10 + PS8 graticule, Jencons Scientific Ltd., Cherrycourt Way Industrial Estate, Stanbridge Road, Leighton Buzzard, Bedfordshire LU7 8UA)

(9) 37^{o}C incubator and 4 C refridgerator.

APPENDIX 2

Reagents from the following sources have been found satisfactory for preparing radial haemolysis gels by Methods I & II

(1) Agarose
(a) Miles Laboratories, PO Box 37, Stoke Poges, Slough SL2 4LY,
(b) Indubiose 37, Uniscience Ltd., 8 Jesus Lane, Cambridge CB5.

(2) Cells
Sheep red blood cells (in alsevers),
(a) Tissue Culture Services, 10 Henry Rd., Slough SL1 2QL.

(3) Antigen
Rubella haemagglutinating antigen (lyophilised, freeze-dried),
(a) Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT.
(b) Flow Laboratories (discontinued).

(4) Complement
Richardsons preserved guinea-pig,
(a) L.I.P Ltd., 111 Dockfield Rd., Shipley, West Yorkshire BD17 7AS.
(b) Tissue Culture Services (address as above).

(5) Diluent Complement fixation buffer tablets,

(a) Oxoid Ltd., Wade Rd., Basingstoke, Hants RG24 0PW.

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Rubella epidemiology in South East England

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SUMMARY

Analyses of data collected in a large survey (sample size > 3000) of rubella antibody in South East England, finely stratified according to age, reveal age-dependent changes in the pattern of virus transmission. The rate or force of infection changes from low in the young children to high in the 5 - to 15-year-olds and back to low again in the adult age classes (there is a 50% reduction between the 5- to 15-year-olds and the 20+-year-olds). Raised levels of immunity are recorded in the teenage and young adult female segments of the population as a consequence of the UK rubella immunization programme. Mean antibody concentrations show a decline with age and are, on average, lower in vaccinated females when compared with unvaccinated males of the same age. The interpretation of horizontal cross-sectional serological data and future research needs are discussed.

INTRODUCTION

Recent studies of the transmission dynamics of rubella virus have highlighted the importance of quantitative information concerning age-specific rates of infection to any detailed assessment of the impact of mass immunization on the incidences of rubella and congenital rubella syndrome (CRS) (Anderson & May, 1982*a*, 1983*a*, 1984, 1985; Schenzle, 1984; Anderson, Grenfell & May, 1984; Anderson & Grenfell, 1986; Grenfell & Anderson, 1985). Advances in the development of a mathematical framework to aid in the design of immunization programmes have, to some extent, outstripped the acquisition of the relevant epidemiological data needed to compare prediction with observation. The combination of these theoretical and empirical components is required to assess the likely impact of different immunization policies.

Changes in the force of infection (the per capita rate at which susceptibles acquire infection per unit of time) with respect to age are of particular significance in this respect (Anderson & Grenfell, 1986; Grenfell & Anderson, 1985). Past attempts to assess the level of vaccine coverage required either to eliminate rubella, or to reduce the incidence of CRS to a defined level, have been based on the

assumption that the force of infection, λ, is constant and independent of age (Knox, 1980; Dietz, 1981; Anderson & May, 1982*a*, *b*; 1983*b*;Hethcote, 1983). Recent research has begun to explore ways of modifying this assumption in the light of data compiled from case notification records and horizontal serological surveys (Anderson & May, 1985). These studies suggest that the force of infection adopts low values in young children, rises to a peak value in teenagers, and then declines to a low value in adult age classes (Anderson & May, 1983*a*, 1985; Anderson & Grenfell, 1986). Heterogeneity in mixing of individuals, both within and between age classes, is thought to be responsible for observed trends.

This paper reports the results of a horizontal cross-sectional seroepidemiological survey of rubella antibodies in samples of sera collected from males and females in a wide range of age classes between 1980 and 1984 in South East England. The survey was motivated by (i) the paucity of published serological data on rubella infection in male and female populations finely stratified according to age, (ii) the need to assess the impact of the current UK vaccination policy on the prevailing levels of herd immunity to infection, and (iii) the need to assess whether the force of infection varies systematically with age.

METHODS AND MATERIALS

Serum samples

Serum samples were collected from two sources. The Department of Virology, King's College Hospital, London, provided aliquots of 1556 sera (age range of patients, 0–93 years) that had been submitted for diagnostic tests and stored at −20 °C since 1980. The Blood Transfusion Centre for South East England provided 1822 serum specimens from blood donors (age range of donors 18–65 years) collected during 1984. The catchment area for both sources of material is, in broad terms, South East England.

Radial haemolysis (RH) test

The RH test for rubella-specific IgG antibody described by Kurtz *et al.* (1980) was used with the following modifications:

Reproducibility of results. Wells of 3 mm diameter and capacity of 7–8 μl were filled with test sera dispensed from haematocrit tubes. Gels were incubated for 24 h. Test to test variation was monitored by determining the antibody concentration of five control sera in each test series.

Quantification of antibody concentration. A local standard serum specimen was calibrated by parallel line assay (Finney, 1978) with a British Standard Serum containing 360 international units (i.u.) of rubella antibody (obtained from the National Institute of Biological Standards and Control, Holly Hill, London). For each gel in a test series, serial two-fold dilutions of the local standard in antibody-negative serum, from undiluted to 1 in 128, were tested in randomly located wells, and the equation of the linear relationship between haemolysis zone diameter and logarithm of antibody concentration (i.u./ml) calculated by least-squares regression. All zones of haemolysis were measured to the nearest 0·1 mm using an eyepiece lens with graticule. The antibody concentration of each test serum was derived from the linear equation of standard serum dilutions. Sera with

concentration of rubella-specific antibody equal to or greater than 3·3 i.u./ml were regarded as positive.

Inter-serum interference. The incidence of overlapping zones of haemolysis was reduced by cutting only 49 wells, in hexagonal configuration, per plate. Where haemolysis zones overlapped preventing accurate measurement the sera were retested.

Sera containing anti-sheep red blood cell antibody. A serum producing zones of haemolysis of equal diameter with both antigen coated and uncoated sheep red blood cells (SRBC) and greater than the test zone for the 3·3 i.u./ml standard may contain rubella-specific antibody, the detection of which is obscured by the lysis caused by anti-SRBC antibody. Sera falling into this category were incubated in SRBC for 1 h and retested.

Validation of negative and equivocal RH results. All sera giving negative results on initial testing were retested by RH. Two separate, randomly selected samples of antibody-negative sera each comprising some 10% of the total number of negative sera, were tested by haemaglutination inhibition (HI) (PHLS Monograph No. 16) and by the Rubazyme enzyme-linked immunosorbent assay (ELISA) (used according to the instructions of the manufacturer, Abbott Diagnostics, UK) to determine the incidence of 'false negative' RH results. All sera giving zones of atypical lysis were tested by HI or ELISA.

Quantitative methods

Age-specific forces or rates of infection ($\lambda(a)$) were estimated from the age-stratified serological data (proportions seropositive in each age class) by the method recently described by Grenfell & Anderson (1985). The technique is based on a 'polynomial catalytic infection model' of the rate at which the proportion seropositive changes with age, and employs maximum-likelihood methods in estimating the age-specific forces of infection (Griffiths, 1974; Grenfell & Anderson, 1985). In the model, the proportion who have experienced infection by age a, $F(a)$, is defined as

$$F(a) = 1 - \exp\left[-\int_0^a \lambda(\alpha) d\alpha\right]. \tag{1}$$

Here $\lambda(a)$ denotes the age-specific rate of infection. The parameter $\lambda(a)$ may be set at zero below some lower age limit to denote the duration of protection against infection induced by maternally derived antibody. The function $\lambda(a)$ is expressed as a polynomial of order k, where

$$\left.\begin{aligned} \lambda(a) &= \sum_{i=0}^{k} b_i a^i \quad (D < a \leqslant U), \\ \lambda(a) &= 0 \qquad\quad (a \leqslant D). \end{aligned}\right\} \tag{2}$$

Here U denotes the upper age limit of the data, D the duration of maternal antibody protection, and the b_i's represent the coefficients of the polynomial function. A number of assumptions are incorporated in the model which influence the accuracy of the estimation method for the age-specific forces of infection. They are as follows: (i) the infection is assumed to be endemic and at some quasi-equilibrium state within the population (this state may be oscillatory in nature); (ii) the infection is assumed to induce lifelong immunity to reinfection and not to

Table 1. *Reproducibility of the radial haemolysis test*

	Controls				
	1 (High)	2	3	4	5 (Low)
Mean (ln(i.u./ml))	6·98	6·23	4·43	3·97	3·17
n	7	7	10	11	10
$*s^2$	0·007	0·017	0·014	0·017	0·024
CL	0·080	0·118	0·086	0·088	0·110
CV %	1·23	2·09	2·71	3·31	4·90

* Test for homogeneity of variance – no significant inhomogeneity at 1 % level.
n, sample size, s^2, variance, CL, confidence limit (95 %) and CV %, coefficient of variation.

increase the mortality of infected individuals; (iii) it is assumed that horizontal changes in the proportion seropositive mirror longitudinal changes for specific cohorts of individuals. Assumptions (i), (ii) and (iii) broadly hold for developed countries provided the inter-epidemic period of the oscillations in disease incidence (4–5 years for rubella, see Anderson & May, 1983*a*; Anderson, Grenfell & May, 1984) is short in relation to the age span over which serum samples are collected (0–75+ years in this study).

The mean age, A, at which susceptibles acquire infection can be estimated directly from eqns. (1) and (2), where

$$A = D + \int_D^\infty [1 - F_{(a)}]da, \tag{3}$$

and D denotes the average duration of protection provided by maternal antibodies. The inter-epidemic period, T, of directly transmitted viral and bacterial infections that induce lifelong immunity, is related to the average age at infection (see Anderson & May, 1982*a*, 1983*a*), where

$$T \simeq 2\pi(AK)^{\frac{1}{2}}. \tag{4}$$

Here, K is the sum of the latent and infectious periods of the infection. For rubella, the period T is approximately 4–5 years.

Oscillatory fluctuations in the incidence of infection may well be reflected in horizontal age-stratified serological profiles, both with respect to the proportions seropositive and to the mean antibody concentrations in each yearly cohort of people. Time series analyses (auto-correlation and spectral analysis) were employed to examine the data for regular oscillatory fluctuations in seropositivity and mean antibody concentrations within the male segment of the sample population (for a discussion of the statistical techniques, see Anderson, Grenfell & May, 1984). No significant correlations were found.

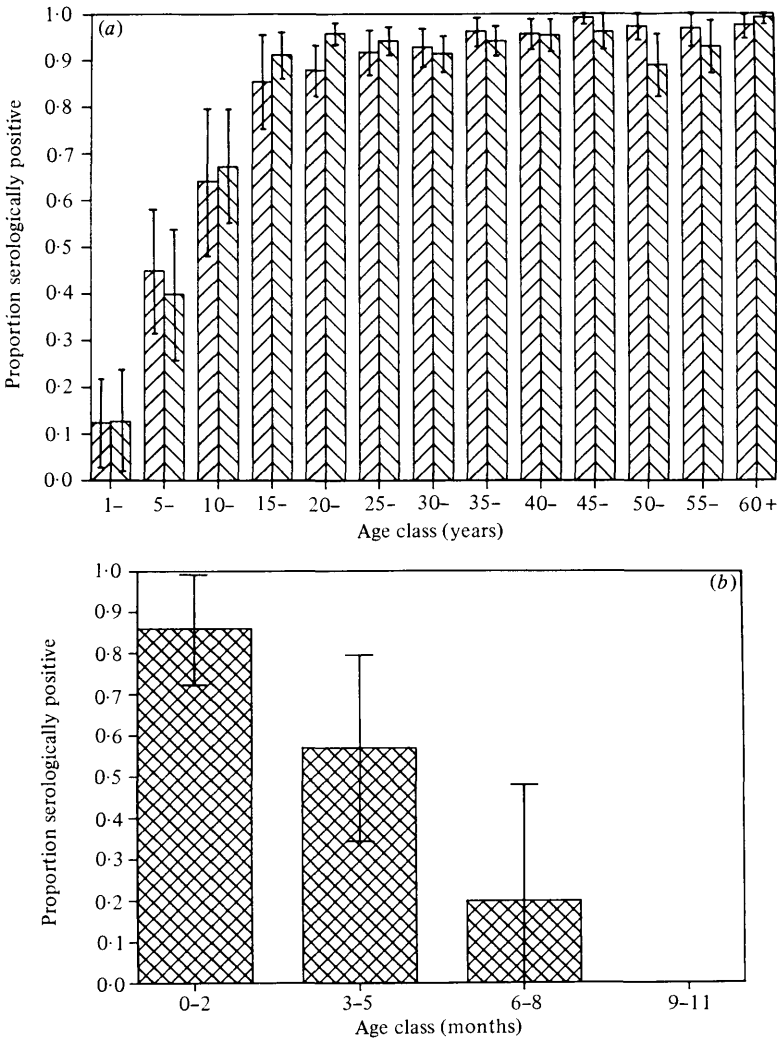


Fig. 1. Graph (*a*). Serological profile for males (▨) and females (▧) stratified according to age. The vertical lines at the top of the bars denote 95 % confidence limits (based on the positive binomial distribution). (Sample sizes, for males and females respectively, are as follows: 1–4, 48, 39; 5–9, 60, 50; 10–14, 39, 61; 15–19, 48, 137; 20–24, 141, 310; 25–29, 146, 259; 30–34, 169, 235; 35–39, 163, 207; 40–44, 138, 151; 45–49, 126, 103; 50–54, 110, 100; 55–59, 93, 84; 60+, 124, 127).

Graph (*b*). The decay in maternally derived antibody (+95 % confidence limits) over the period from birth to 11 months of age. (Sample sizes, 0–2, 28; 3–5, 21; 6–8, 10; 9–11, 7.)

RESULTS

Sensitivity and reproducibility of the RH test

The local standard serum when diluted 1 in 64 in antibody-negative serum to give a preparation containing 3·3 i.u./ml was the highest dilution that consistently gave a visible zone of lysis. This degree of sensitivity is in accordance with previous reports (Neumann & Weber, 1983; Morgan-Capner, 1984). Inter-test variation in results is shown in Table 1. The variance in antibody concentration was found to

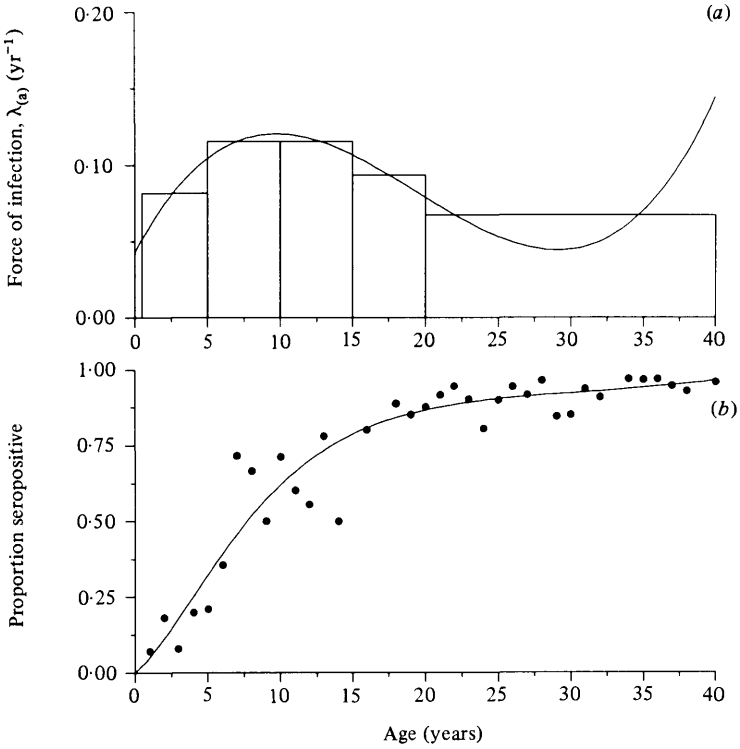


Fig. 2. Estimates of the age-dependent forces of infection derived from the serological data for males in SE England (1980–4). Graph (*a*) records the (maximum likelihood method) estimated values of the force of infection, $\lambda(a)$ (yr^{-1}) based on the polynomial catalytic model of Grenfell & Anderson (1985). The polynomial fit derived from the data is shown as a curved line while the histogram bars denote the average values (derived from the polynomial) for various age classes. Graph (*b*) compares the predicted change in the proportion serologically positive with age (——) based on the $\lambda(a)$ estimates defined in graph (*a*), with observed data (●).

be constant and independent of the mean antibody level. Consequently, the coefficient of variation (CV %) was inversely related to the mean antibody concentration. The mean CV % (weighted by sample size) is 3·0, which is similar to that found by Neumann and Weber (1983) in their evaluation of the RH test.

The retesting of 20 % of the RH negative sera by HI and ELISA gave no discrepant results, reinforcing the general observation that false negatives are rarely encountered in RH screening (Morgan-Capner, 1984). However, from the total 3378 sera tested, two were found which inhibited haemolysis by sera tested in adjacent wells; in both these sera rubella-specific IgG was detected by ELISA.

Prevalence of rubella antibody

The proportions of males and females seropositive for rubella in each five-year age class are shown in Fig. 1 (*a*). The slower rate of acquisition of antibody by males between the ages of 10 and 29 years is indicated by the lower proportions of seropositive males than females. For the age class 20–24 years the difference is significant at the 5 % level (assuming binomial distribution of seropositivity).

Table 2. *Estimates of various epidemiological parameters of rubella infection derived from a serological survey of SE England*

Data	Average age at infection* A (years)	Basic reproductive† rate of infection, R_0	Inter-epidemic‡ period, T (years)
Males	10·83	6·78	5·10

* Estimated by a maximum likelihood method (see Grenfell & Anderson, 1985).

† R_0, $L/(A-D)$ where L, life expectancy; A, average age at infection; and D, average duration of maternal antibody protection (= 0·5 yr) (see Anderson & May, 1983*a*).

‡ $T = 2\pi(AK)^{\frac{1}{2}}$ where K = sum of latent plus infectious periods (= 22 days) (see text).

Seropositivity in females between 10 and 29 years old reflects antibody acquired by both natural infection and immunization; the contribution of vaccine-induced antibody raises the proportion seropositive in the 20–24 years age class to 95%, a level not attained in males until the 35–39 years age class.

Fig. 1(*b*) shows the decline of maternally derived antibody. The average persistence of detectable maternal antibody levels is between 3 and 6 months; antibody was not detectable in any of the seven sera from infants aged 9–11 months.

Age-dependent changes in the force of infection

Changes in the true force of infection (λ) with respect to age can be estimated from the serological profile only within the male segment of the sample population since the relative contributions of vaccination and natural infection to female immunity cannot be determined. Changes in λ with respect to age, estimated from the serological data by the maximum-likelihood method described by Grenfell & Anderson (1985), are recorded in Fig. 2. Mean values of the force of infection over selected age intervals (0·5–5·0, 5–10, 10–15, 15–20, 20–40 years) and the best fit polynomial catalytic model (to the observed serological data) are displayed in Fig. 2(*a*). Using the data from male subjects, and assuming the duration of maternally-derived protection to be 6 months (Fig. 1(*b*)), the mean age at infection, A, is estimated to be 10·8 years (Table 2). This estimate is somewhat higher than that quoted previously (see Anderson & May, 1985) from smaller studies and indicates that immunization of 10- to 15-year-old girls in the period 1970–1984 may have caused a slight reduction in the net force of infection.

Rubella antibody concentrations

The frequency distribution of antibody concentrations (i.u./ml) for seropositive individuals of all ages was found to exhibit a marked positive skew. A logarithm ($\log_e$) transformation of the raw data was therefore employed in the parametric statistical tests used to examine changes in mean concentrations with age and sex.

Mean antibody levels show a significant decline with age (regressions of mean concentration (both males and females) against age gave negative slopes ($P < 0{\cdot}005$)) as recorded in Fig. 3. Similar trends have been noted in age-stratified serological surveys for measles (Black, 1959) and mumps (Wagenvoort *et al.* 1980) antibodies. Over the age range, 13–27 years, influenced by the UK rubella vaccination policy started in 1970, there is a statistically significant difference (*t*-test

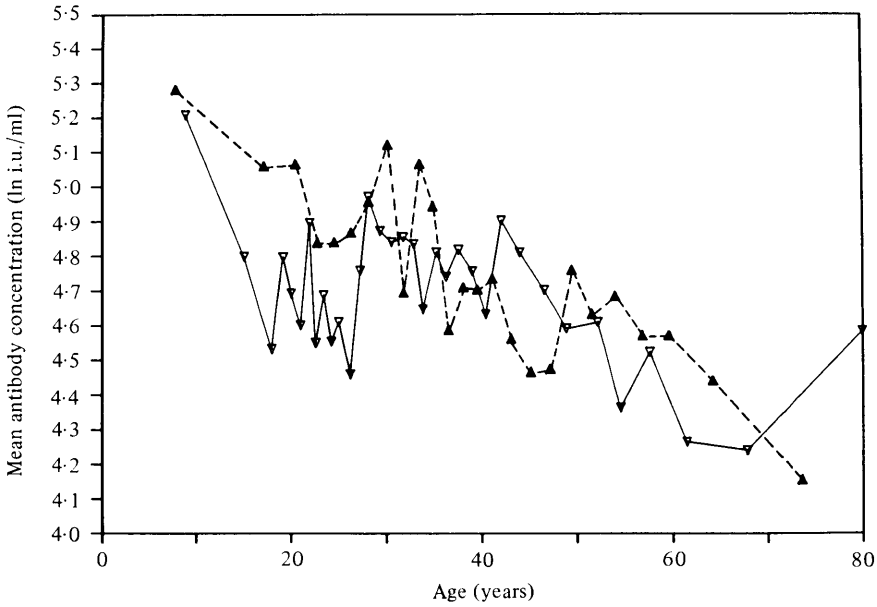


Fig. 3. The decay in mean antibody concentration with the age and sex of the individual from whom the serum sample was collected ▲---▲, males; ▽—▽, females). (Each point denotes an average value for 50 samples excepting females of age 80 (n = 29) and males of age 74 (n = 46).)

on $\log_e$-transformed data $P < 0{\cdot}005$) in the mean antibody levels in males and females. The latter have a lower average antibody level (that could not be attributed to haemodilution in the samples obtained from pregnant women), which suggests that vaccination induces lower levels of antibody production than those stimulated by natural infection. This hypothesis is supported by a recent comparison of antenatal patients in 1975–6 with those in 1978–9 (CDR, 1984) which shows an overall decrease in the number of females with high antibody levels over the time interval between the two sample points (see also Enders, 1985).

The variability in antibody concentration within a given age group shows an overall tendency to increase with age (Fig. 4). This increase suggests that the probability of falsely identifying an individual who has experienced infection as seronegative (i.e. < 3·3 i.u./ml) increases with age (Fig. 5). As such, the estimated force of infection in the older adult age classes is likely to be an underestimate.

DISCUSSION

In the absence of mass immunization, age-stratified serological profiles provide important information on the force or rate of infectious disease transmission within large communities of people. Differences in seropositivity between two age classes provide quantitative information on the per capita rate at which susceptibles acquire infection over a defined interval of time. If the proportion seropositive rises rapidly with age, the force of transmission is high and the converse also applies. Within a population in which the infection is endemic, and at a quasi-steady state (which may be oscillatory in nature), age-related changes reflect time-dependent

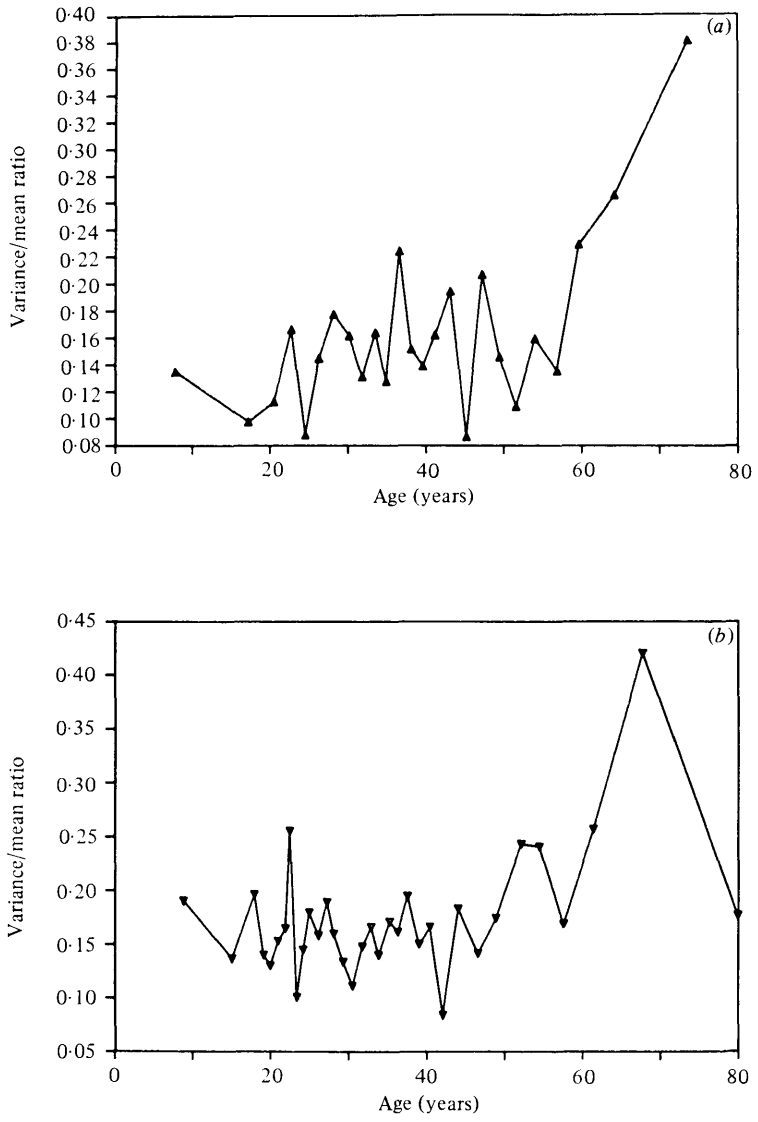


Fig. 4. Change in the variance of antibody concentration per samples of sera collected from patients in different age and sex classes. The change is recorded by the variance to mean antibody concentration ratio. Note how the ratio increases in the older adult age classes. Graphs (*a*) and (*b*) record changes in the male and female segments of the population sample respectively. (Sample sizes as for Fig. 4.)

rates of transmission (Muench, 1959; Anderson & May, 1982*a*, 1983*a*; Grenfell & Anderson, 1985). In other words, horizontal changes reflect longitudinal trends.

The present study was undertaken to provide a reliable data base for the study of rubella transmission in the UK prior to immunization (by surveying seropositivity levels in children less than 10 years old, and male subjects) and to examine the impact of the current vaccination policy. Previous studies have sought to obtain this information, either from case notification records or from small scale serological surveys of restricted age groups (e.g. Field, 1967). Serological data are likely to

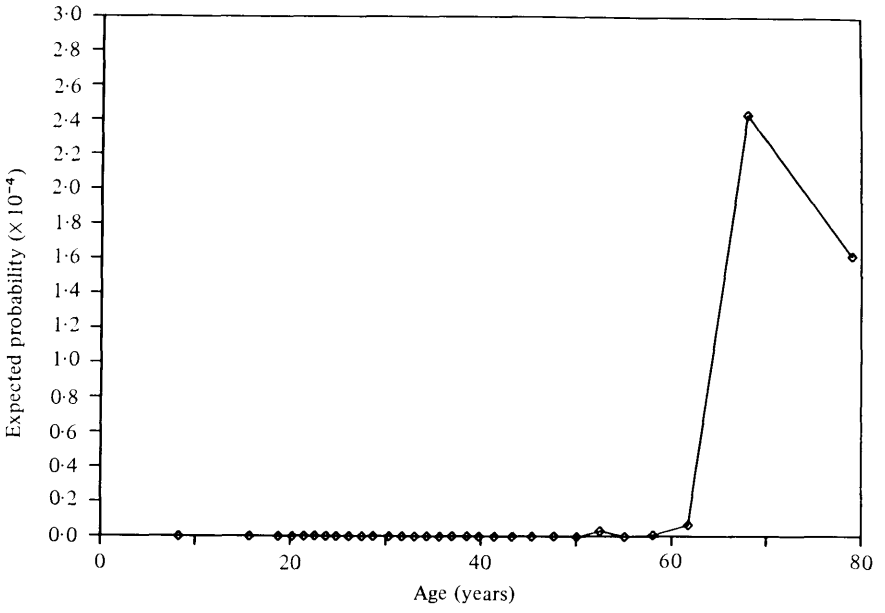


Fig. 5. Changes in the probability of falsely identifying a seropositive individual as seronegative (given a test sensitivity of $\geqslant 3{\cdot}3$ i.u./ml of antibody) with age. The calculations are based on the assumption that antibody concentrations within an age class are normally distributed in seropositive individuals with means as defined in Fig. 4. Sample sizes are 100 sera (males and females) per average excepting for age 78 where sample size was 50.

provide a truer reflection of rubella epidemiology for two reasons: first the clinical diagnosis of rubella is notoriously difficult (see for example Anderson, Kidd & Morgan-Capner, 1985; Cohen & Shirley, 1985) and second there is considerable age-dependent bias in reporting of cases. However, serological data must also be interpreted with care.

The serological profiles recorded in Fig. 1(*a*) show that levels of immunity in the older adults (50+-year-olds) never attain 100%. In part this is a consequence of the low force of infection in the adult classes (see Fig. 2). If an individual escapes infection during the child and teenage years, the probability of acquiring rubella infection in the adult years appears to be low (probably as a consequence of behavioural factors). However, this observation is based on the estimates of the forces of infection which are derived from the serology itself. If the serological data is not a true picture of past experience of infection in those where the interval between infection and serological testing is long, then the estimates of the force of infection are inaccurate. Seronegativity in adults could be due to other factors such as genetic determination of antibody production following infection (and perhaps susceptibility to infection) or the inability to detect low antibody levels in individuals where the interval between infection and test is long (many decades). Some evidence in support of the latter conjecture is provided in Fig. 3, which records a decay in the mean antibody concentration of seropositives with an increase in age. The rate of decay itself may be determined by genetic factors since the variance in antibody concentration among seropositive individuals also rises with age (Fig. 4). This issue requires further research. Preliminary studies could

profitably focus on assessing the degree of association between HLA type and antibody titre in adult age classes. Evidence for an association between rubella antibody titres and HLA type in vaccinated individuals provides some support for this line of enquiry (Spencer *et al.* 1977; Kato *et al.* 1982). However, it is important to note that although serum antibodies are highly correlated with immunity they may not necessarily be the major mediators of acquired immunity to viral infection (Roitt, 1984). Cell-mediated factors are thought also to be important.There are recorded instances of seronegative individuals resisting infection or reinfection with mumps virus, for example in the Netherlands (Wagenvoort *et al.* 1980). This may be genetically linked and implies either that low antibody titres (undetectable by current methodology) provide adequate protection against infection, or more likely that cellular immunity is of greater importance in suppressing infections.

The present study suggests, in accord with others (O'Shea *et al.* 1982, 1984, 1985), that antibody levels in vaccinated individuals are on average lower than those in naturally infected individuals; the mean concentrations in teenage and young adult females are lower than in males of the same age (Fig. 3). The degree of immunity to rubella infection provided by low concentrations of antibody is unclear. In the present study a low threshold (3·3 i.u./ml) was regarded as indicative of immunity since challenge studies have revealed that low levels of naturally acquired antibody confer immunity to reinfection (Mortimer *et al.* 1981; O'Shea *et al.* 1982, 1983, 1984, 1985). However, it must be remembered that RH does not distinguish between antibody produced in response to natural infection and that produced in response to vaccination, and similar challenge studies suggest low levels of vaccine-induced antibody do not correlate well with protection from reinfection (Balfour *et al.* 1981; Harcourt *et al.* 1980). Thus the value of long-past vaccination in preventing rubella infection must be in doubt. If vaccine-induced immunity is not life-long it will be of advantage to continue the present UK policy since to protect the unborn child the vaccine needs only to be effective for a maximum of a few decades.

The selective rubella immunization policy practised at present in the UK, vaccinating only girls between the ages of 10 and 15 years, and adult women found to be seronegative during pregnancy in the postpartum period, was designed to have little impact on the net force of virus transmission within the younger segments of the population. A small but discernable effect was, however, detected in the present study where the mean age at infection calculated was slightly higher than has previously been recorded (see Anderson & May, 1985). This slight reduction in the force of infection consequent upon immunization means that the use of serological data from male subjects to examine the dynamics of rubella transmission prior to immunization may be slightly inaccurate, underestimating the true natural force of infection.

The general pattern of change in the rate of antibody acquisition with age (low in young children, high in older children and early teenagers, falling to low again in adults), recorded in this survey (Fig. 2(*a*)) is similar to that observed for other predominantly childhood infections such as measles, mumps and chickenpox (see Anderson & May, 1985). The factors inducing age-related changes in transmission are not fully understood although heterogeneity in the degrees of contact and mixing between different age classes are likely to be important (Schenzle, 1984;

Anderson & May, 1983*a*, 1984, 1985). The high forces of infection in the 5- to 15-year-olds may be assumed to be due to high contact rates among children attending school. An observed value of the force of infection within a given age class is a composite measure of the rate at which susceptibles in that age class acquire infection from infectious individuals in all age classes. Patterns of contact and mixing between age classes are likely to vary in different societies and this in part explains the observed differences in the rate at which antibody is acquired in different age classes in the developed and developing world. For example, the proportion seropositive rises more rapidly with age in large urban communities in areas such as Russia (Kantarovich *et al.* 1983) and The Gambia (Clarke *et al.* 1980) than is seen in the UK and USA (Anderson & May, 1983*a*). In addition to these behavioural factors it should be noted however that demographic parameters such as the net birth rate, which determines the input of new susceptibles into a community, are also important (Anderson & May, 1985).

Our estimation of age-dependent changes in the force of infection is based on horizontal serological data. As such it provides no information on short term longitudinal changes such as those induced by seasonality in virus transmission. Seasonal changes in the incidence of childhood viral and bacterial infections may arise as a direct consequence of the influence of climatic factors on virus transmission or, more significantly, as a consequence of changes in human behaviour related to season (Anderson, Grenfell & May, 1984; Schenzle, 1984; Fine & Clarkson, 1982; Yorke & London, 1973). It is possible that the latter factor, particularly with respect to school children, alters the pattern of infection within and between age classes. Investigation of this problem would require detailed age-stratified serological data based on sequential serum sampling at intervals throughout a year. Such samples are not available at present but this problem deserves further attention in future research.

The differences between the male and female segments of the population observed in the present study illustrate the impact of immunization of females between the ages of 10 and 15 years, and are in agreement with a recent study conducted in the Manchester area in England (Miller *et al.* 1985), and the longitudinal studies by Clarke *et al.* (1983). In the present study the degree of herd immunity in adult women was not detectably increased by the 'second arm' of the UK vaccination policy, the postpartum immunization of women found to be seronegative at antenatal booking. Recent research has revealed that predictions of the levels of vaccination coverage required to eradicate common infections which are based on mathematical models assuming the force of infection to be constant, tend to produce higher estimates than models which take account of the observed patterns of change in λ with age (Schenzle, 1984; Anderson & May, 1984, 1985). In other words, predictions based on homogeneous mixing models give too pessimistic a picture of the levels of vaccination coverage required for elimination of a virus from large populations. The results obtained in the present study do not entirely support this view since the observed decline in λ between the teenage and adult age groups is relatively small. Certainly the decline in λ in the adult (20- to 40-year-olds) age classes is less marked than has been reported for measles and pertussis.

However, the observed decay in the force of infection from a value of 0·115 per year in the 5- to 15-year-olds to 0·067 per year in the 20+ age group, a reduction

of almost 50%, is still likely to have a substantial impact on predictions of the changes in the incidence of rubella infection compared with those obtained from models assuming transmission to be constant and independent of age. The impact of these data upon design of vaccination programmes for the control of rubella and prevention of congenital rubella infection are the subject of an accompanying paper (Anderson & Grenfell, 1986).

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