STUDIES ON THE EPIDEMIOLOGY AND PREVENTION OF RUBELLA

Summary

The work for this thesis was conducted between December 1965 and December 1968 at the Epidemiological Research Laboratory, Central Public Health Laboratory, Colindale Avenue, London, N.W. 9.

The aims of the studies undertaken were as follows:

1. To assess the proportion of women of child-bearing age resident in different regions of the United Kingdom who possess antibody to rubella with special reference to their age-group, country of origin, previous history of rubella and obstetric experience.

2. To observe the differences in rubella antibody titre of the various batches of immunoglobulin which are in current use in the United Kingdom and to determine, by means of a controlled trial, the protective effect of immunoglobulin of high rubella antibody titre when given to contacts of rubella who are within the first sixteen weeks of pregnancy.

3. To investigate the infectiousness of the rubella virus in the home, in a semi-residential community and on casual contact with patients suffering from the disease. In addition the proportion of subclinical and second attacks of rubella that can be verified by laboratory means were noted.

Although several investigations are described in the thesis, each

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is intended to provide some information on the risk of infection to persons, especially if in early pregnancy, when they are exposed to the rubella virus and to determine the protection they may expect if given immunoglobulin after contact with the disease. Because it is now possible to isolate the rubella virus and perform antibody titrations, the results of each study was based on laboratory findings.

The investigations were carried out with the help of general practitioners, doctors in charge of ante-natal clinics and virologists at the laboratories of the Public Health Laboratory Service.

RESULTS

Immunity to Rubella

1. The majority of persons investigated already possessed antibody to rubella by the time they reached adult life. The proportion immune varied in the different geographical areas studied and ranged from 96 per cent of the women from Leeds and Keighley to 80 per cent of those from London. Taking all the regions into consideration 91 per cent of the women examined were immune.

2. Immunity to rubella varied to some extent with age. Of those less than 20 years, 84 per cent were immune compared with over 90 per cent in older women.

3. Among the immigrant population living in the United Kingdom, the African and West Indian populations appeared to have a smaller proportion of persons who possessed rubella antibody.

4. The reported history of an attack of rubella did not agree with the patient's immunity to rubella.

5. Women without children were more likely to be susceptible to rubella than women with families.

6. A large proportion (97per cent) of the young adult males investigated also possessed antibody to rubella.

Immunoglobulin

7. Ampoules from fifteen batches of British immunoglobulin were tested for neutralising antibody to rubella. All contained

antibody, the titres ranging from 1 : 60 to 1 : 320.

Attack rate in rubella contacts given immunoglobulin

8. (a) When no account was taken of the immunity of pregnant women in contact with cases diagnosed clinically as having rubella, only 1.4 per cent developed an illness with a rash after having had an inoculation of 750 mg. immunoglobulin.

(b) When virological studies were performed, however, and the analysis restricted to susceptible pregnant women who were in home contact with a case of rubella proved by isolation of the virus, then 80 per cent became infected either clinically or subclinically despite having had 750 mg. immunoglobulin.

9. Even when the dose of immunoglobulin was increased to 3,000 mg., four of six susceptible women developed the disease after contact with a confirmed case of rubella.

10. The risk of contracting rubella was much less if the index case was not a member of the same household. The attack rate for inoculated, susceptible pregnant women in contact outside the home with a confirmed case of rubella was a quarter of the rate found for home contacts.

Attack rate in uninoculated contacts of rubella

11. Fifty per cent of uninoculated susceptible women of childbearing age developed rubella after home contact with a virologically proved case. As 80 per cent of pregnant women became infected, pregnancy may increase susceptibility.

12. In the Hendon Police College - a residential community - 100 per cent of those cadets who were susceptible developed rubella after the introduction of infection into the college.

Second attacks of rubella

13. Second attacks of rubella were found to be rare. Only one of 106 immune pregnant mothers developed a second attack of rubella following exposure. The clinical diagnosis was verified by virus isolation but neither in this woman nor the 105 others did a four-fold rise in antibody titre occur.

Subclinical attacks

14. (a) The number of subclinical attacks of rubella in the communities studied was probably considerable as judged by the proportion (88 per cent) of women who possessed antibody to rubella but who did not give a past history of clinical infection.

(b) Eleven per cent of uninoculated women and 14 per cent of uninoculated men who developed rubella had a subclinical infection.

(c) Forty-eight per cent of the women who developed rubella after immunoglobulin had a subclinical infection. Immunoglobulin, therefore, may suppress the clinical features of the disease but not prevent infection.

Isolation of the rubella virus

15. Rubella virus was isolated in about 50 per cent of specimens obtained from cases of rubella during the first three days after

the onset of the rash. During the next three days the virus was isolated from about 25 per cent of cases; thereafter it was seldom isolat

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12th March 1969.

Professor C. M. Fleming,
Dean of the Faculty of Medicine,
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Dear Professor Fleming,

Studies on the Epidemiology and Prevention of Rubella

I should like to submit the attached thesis for consideration for the degree of M.D.

Acknowledgment has been made in the thesis to the help given by others, but the organisation of the investigations described, the analysis of the results and the composition of the thesis have been done by myself.

Yours sincerely,

D. Reid

D. Reid.

P.S. Fee attached.

STUDIES ON THE EPIDEMIOLOGY AND PREVENTION OF RUBELLA

by

Daniel Reid

M.D. Thesis submitted to the

University of Glasgow

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PART I

INTRODUCTION

CHAPTER 1

THE BACKGROUND AND AIMS OF THE THESIS

The clinical features of rubella have been recognised for at least three centuries without exciting much medical interest but during the last thirty years three major advances have completely changed our understanding of the epidemiology of the disease and the importance of its prevention.

Firstly, in 1941 Dr. Norman M. Gregg recognised that a woman who developed rubella during early pregnancy was liable to give birth to a malformed infant. Secondly, it was realised that passive immunisation with either pooled plasma or human immunoglobulin (gamma globulin) might protect the pregnant mother who was in contact with a patient suffering from rubella during the period when foetal malformation was most likely to occur, i.e. the first sixteen weeks after conception. As immunoglobulin is that fraction of the plasma containing antibody it was hoped that by immunisation of the mother, maternal viraemia would be prevented and, in turn, the developing foetus safeguarded. The increasing use of immunoglobulin gave rise to many investigations to determine its protective effect in pregnant rubella contacts. Thirdly, in 1962 the isolation of the rubella virus was reported (Weller and Neva, 1962; Parkman, Buescher, and Artenstein, 1962). This rapidly led to the

establishment of the laboratory diagnosis of rubella both by virus isolation and antibody titration. Prior to this, the study of the disease was hampered by the mildness of the clinical signs and the similarity of the features to those produced by other infections; a confident diagnosis was therefore difficult. Many patients could not recall whether they had had rubella in the past, and, because of this, a previous history was not a reliable basis on which to distinguish persons susceptible to the disease (i.e. not previously infected) from those who possessed immunity. Moreover, subclinical attacks could not be detected nor could the rubella antibody titres of different batches of immunoglobulin be estimated. After the discovery of the virus however, it became possible to determine whether or not a person possessed antibody to rubella and to undertake epidemiological studies to examine the infectiousness of the virus, the incidence of subclinical, and of second attacks of rubella. The protection afforded by immunoglobulin, when given prophylactically to pregnant contacts of the disease, could also be more accurately assessed because information on such factors as the susceptibility of the pregnant woman, the diagnosis of rubella in the patient with whom she was in contact, the development of infection (either clinical or subclinical) and the rubella antibody titre of the immunoglobulin could now be

obtained. In addition, the isolation of the rubella virus made possible the production of vaccines which should ultimately diminish the risk to pregnant women of developing the disease after contact with a case.

In the light of these laboratory advances the Public Health Laboratory Service in England and Wales set up a Working Party in December 1965 to re-assess the protective effect of immunoglobulin when given to contacts of rubella during the first sixteen weeks of pregnancy. As secretary of this Working Party I was responsible for the planning and organisation of the various epidemiological investigations and for the execution of much of the field work together with the analysis of the results. The majority of the investigations described in this thesis were made under the auspices of the Working Party but some were also conducted independently of it.

The aims of these studies were as follows :-

1. To assess the proportion of women of childbearing age, resident in different regions of the United Kingdom who possess antibody to rubella with special reference to their age-group, country of origin, previous history of rubella and obstetric experience.
2. To observe the differences in rubella antibody titre of the various batches of immunoglobulin which are in current

use in the United Kingdom and to determine, by means of a controlled trial, the protective effect of immunoglobulin of high rubella antibody titre when given to contacts of rubella who are within the first sixteen weeks of pregnancy.

3. To study the infectiousness of the rubella virus under various circumstances and to note the proportion of subclinical and second attacks of rubella that can be verified by laboratory means.

Although several investigations are described in the thesis each is intended to provide some information on the risk to persons, especially if in early pregnancy, when they are exposed to the rubella virus and to determine the protection they may expect if given immunoglobulin.

Several aspects of these investigations have already been published elsewhere.* In this thesis the material has been developed, extended and considered in relation to previous studies.

* See attached reprints.

CHAPTER 2

AN HISTORICAL REVIEW

a) Early observations

The first description of rubella was probably made by Daniel Sennert of Wittenberg in 1619 (Goodall, 1934). He considered that the disease, which he termed Ritteln or Rotteln, was related to measles but was less dangerous. In 1676 James Cooke further emphasized the comparative mildness of the disease by referring to "rubeolae which happens to persons in health". Another early reference to rubella was quoted by Fuller (1730) from Pechlin (1671) who described "a small sort of Measles, called Rothel which in his Travels he observed over-running the Palatinate and Swabia sparing no Sex nor Age. Most of them had Restlessness, Lassitude, intense Heat, Loss of Appetite. Some were confined two or three days to their Bed; some that were of foule Bodies, longer but some not at all. Upon taking a Sudorific generally all went off easily and few dy'd of it. It was so rife and contagious that in the City of Stutgard seven hundred lay ill of it at once".

During the eighteenth and nineteenth centuries many people did not consider rubella to be a separate disease.

Gruner (1774) maintained that it was identical to scarlet fever while according to Hebra (1866) Schönlein in 1832 suggested that rubella possessed characters common to both scarlet fever and measles. This view was supported by Copland (1858) who noted that it was "doubtful whether or not this (rubella) should be viewed as a distinct or specific form of disease or merely a variety of either measles or scarlet fever in which many characters of either the one or the other predominate", and by Sir William Aitken (1864) who wrote "In truth it seems to be a hybrid disease developed from the combined poisons of the two fevers".

Commencing with Selle (1788), who considered that "rubella is different from measles in that the fever does not present with bleariness of the eyes and coughing but for the most part with a stiff neck" the concept of a separate exanthem was gradually introduced. Further evidence was given by Willan (1813) who described the disease as "rubeolae sine catarrho" noting that patients suffering from this condition were "peculiarly liable to a second attack of measles" and two years later Maton, relating an outbreak of rubella in London in a paper read before the Royal College of Physicians of London stated that "the period intervening between the application of the infectious influence and the commencement of the disease was considerably longer than has

been noticed in scarlatina". Paterson (1840) describing the features of the disease as they presented among his patients in Leith, wrote "that it possessess characters peculiarly its own". Despite this statement, however, it is likely that confusion with scarlet fever still existed as Paterson maintained in the same article, that severe inflammation of the tonsils occurred and that the disease was "often an extremely and rapidly fatal disorder". Richardson (1867) also believed that rubella was a separate entity and thought that it was elicited by the irregular digestion of some particular form of food.

Thus by the mid-nineteenth century a large body of opinion considered rubella to be unrelated to either measles or scarlet fever. However the disease had to await the Seventh International Medical Congress of 1881 before it received general recognition as a separate disease.

While the controversy over the relationship of rubella to other diseases was continuing, much confusion was also caused by the different terminology. The early German workers used the names "Ritteln", "Rotteln" (Sennert, 1619) and "Rötheln" (Pechlin, 1671); this last term was introduced into the United Kingdom by Paterson (1840).

During the eighteenth and nineteenth centuries, other physicians adopted different names for the disease.

Selle (1788) used the term "rubeolae" and Babington (1867) although differentiating the disease from measles, felt that there was sufficient resemblance to suggest the name "rubeola notha or bastard measles". Richardson (1867) further confused the situation by referring to "rosalia idiopathica". It was in 1866, however, that Henry Veale in a paper describing an outbreak of the disease among children and young adults at the Mount Aboo Hill Station in the Presidency of Bombay suggested that "Rötheln is harsh and foreign to our ears" and that "Rubeola notha and Rosalia idiopathica are too long for general use". He "ventured to propose Rubella as a substitute". This name was not a new one as Richard Russel used it to describe "red gum" of infants in 1755 but after Veale's suggestion it came to be accepted in Britain and the United States. However, because of the early interest shown in Germany, the disease was also commonly termed "German measles" in these countries.

b) Clinical features and complications of rubella

The records of the nineteenth and early twentieth centuries indicated that rubella was a mild disease greatly overshadowed by the more lethal illnesses that were then prevalent and the least troublesome of the childhood

infections (Maton, 1815; Veale, 1866; Babington, 1867; Rolleston, 1937). So trivial were the features of the disease considered that an editorial in the Edinburgh Medical Journal in 1940, celebrating the centenary of Paterson's description of the disease, opened "German measles has been in the news of late and we hear of medical officers of health, headmasters, headmistresses, and others clamouring to have it deprived of .... its place as a notifiable disease and relegated to the limbo of the minor exanthemata .... a nuisance rather than an illness". Were it not for Gregg's discovery the following year of the teratogenic effects of the disease there is little doubt that rubella would have continued to be regarded as trivial and self-limiting for as Dudgeon (1967a) points out, it is still a mild disease at least as far as the clinical features are concerned.

A detailed analysis of the signs and symptoms of rubella was made by Young and Ramsay in 1963. They considered that the following features were important for the diagnosis to be made.

1. Mild prodromal symptoms with absence of coryza but with injection of the tonsillo-pharyngeal area associated with infection of the upper respiratory tract.

2. Cervical lymph node enlargement with variable involvement of the suboccipital lymph nodes.
3. Suffusion of the eyes, with the complaint of a sensation of "grittiness" or even photophobia. The authors considered this to be a most important sign.
4. A macular-type rash appearing behind the ears, on the face and rapidly spreading from above downwards. The lesions become confluent and the erythema generalised giving a "peach bloom" appearance.
5. Occasionally petechial lesions on the palate.

In their series of 114 patients with rubella 94 per cent had injection of the tonsillo-pharangeal area, 97 per cent lymphadenopathy, 85 per cent suffusion of the eyes, and 17 per cent palatal petechiae.

In non-epidemic years when the disease is unexpected and because of the mildness of the physical signs, the diagnosis of rubella is difficult without laboratory help. The disease may be confused with measles, scarlet fever, toxic erythematous rashes of drug origin, infectious mononucleosis and E.C.H.O. virus infections. It is not surprising therefore that Young and Ramsay found that the illnesses in over 20 per cent of patients initially diagnosed as rubella were in fact due to other causes.

The complications of rubella are said to be rare (Kilbourne, 1963) but while this appears to be true of children suffering from the disease there are several reports of adults being more troubled by the sequelae. Fry, Dillane and Fry (1962) noted that 11 of their 74 adult patients with rubella complained of arthralgia with swelling of the joints, redness of the skin and pain on movement. Nine of these patients were women. Symptoms of compression of the median nerve in the carpal tunnel were also noted in three women. Gregg (1941) also mentioned that "an unusual number of young adult patients suffered from arthritis and other rheumatic conditions" after having had rubella.

Thrombocytopenic purpura has also been noted by several authors (Gunn, 1933; Warren, Rogliand, and Potsubay, 1946; Green, Balsamo, Giles, Krugman and Mirick, 1965) usually just as the rash is fading. This complication may present with skin haemorrhages, epistaxis, bleeding from the gums or the gastro-intestinal tract. Purpura is seldom seen in otherwise normal children (Dudgeon, 1967b).

A further complication of rubella is encephalitis. Miller, Stanton, and Gibbons (1956) reviewing 80 cases from the literature noted that the average time of onset of encephalitis was four days after the appearance of the rash.

They estimated that the incidence of this complication was one in 5,000 cases but Pampiglione, Young, and Ramsay (1963) describing ten cases occurring during the 1962-63 epidemic in the United Kingdom, suggested that encephalitis was more common. It is from this complication that death may occur and the mortality is high. Dudgeon (1967b) estimated that during the 1962-63 epidemic, 20 per cent of the patients died within three days of developing encephalitis.

c) Epidemiology

(i) Geographical distribution

Rubella has been reported from nearly every country in the world (Wesselhoeft, 1947; Dudgeon, 1967b; Rubella Vaccine Symposium, 1968). It is endemic in the large centres of population whereas in rural or isolated areas the disease may not recur for several years. For example an outbreak of rubella took place in the Pribilof Islands in Alaska in 1940. It was not till 1963 that the disease recurred when children, returning home from school on the mainland, brought the infection back to the community (Brody, Sever, McAlister, Schiff, and Cutting, 1965).

(ii) Periodicity

In contrast to the two year periodicity of measles

rubella usually causes major epidemics every seven to nine years (Ingalls, Babbott, Hampson, and Gordon, 1960; Dudgeon, 1967b). This cycle may be broken however, during times when large numbers of persons are gathered together. Gregg (1941) mentioned that the epidemic which occurred in Australia in 1940 was particularly widespread and severe. At that time there must have been an increased opportunity for dissemination of the disease in view of the mobilisation in that country for the Second World War. Similarly, as Ingalls (1967) pointed out, rubella was endemic in the United States during 1951 and 1952 and again during 1963 and 1964; these periods coincide with the hostilities in Korea and Viet Nam.

(iii) Seasonal behaviour

Because rubella is not notifiable to the Registrar General it is difficult to accurately determine the seasonal behaviour of the disease in the United Kingdom. However, fifty general practitioners in practices scattered over Britain make regular weekly returns of infectious diseases occurring in their patients to the Records Department of the Royal College of General Practitioners. The returns for rubella during 1967 and 1968 have been arranged in a diagramatic form in figures 1 and 2 and show that for these years the highest monthly incidence was in April and May

but an increased number of patients suffering from rubella was noted in January and the numbers did not markedly decrease till July. Kilbourne (1963) and Ingalls (1967) believed that rubella is a disease of the spring months but the figures of the Royal College of General Practitioners suggest that the disease is prevalent for a longer period in the United Kingdom and includes the latter half of the winter.

(iv) Age distribution

Most authors are agreed that rubella is rare in the first six months of life and in persons over 40 years of age. However, the disease has been reported in the newborn (McCracken, 1963) and in a patient aged 82 years. (Simpson, 1940). There is some disagreement about the age at which the majority of infections occur. Ingalls, Babbott, Hampson and Gordon (1960) considered that children of school age had the highest incidence whereas Dudgeon (1967b) noted that the age-distribution of rubella had changed during the past 60 years from being a disease of early childhood to one that chiefly affects young adults. It may be that because of the recognition of the hazard of rubella in pregnancy, the increased severity of the complications in older patients and the disruption caused by

outbreaks in institutions and among nurses and Service recruits, more attention is now paid to the disease when it occurs in young adults.

(v) Sex incidence

Aycock and Ingalls demonstrated in 1946 that up till the age of five years there is little difference in the attack rate of rubella between males and females but thereafter females are more likely to develop the disease than males. This finding was confirmed in a survey undertaken in 1963 by the Epidemic Observation Unit of the Royal College of General Practitioners; in the childbearing period from 17 to 44 years of age the attack rate in women without a previous history of rubella was 5.5 per cent, but only 0.6 per cent in men of the same age. This difference is presumably due to the closer contact of children with their mothers than their fathers but as mentioned in the report of the Royal College of General Practitioners the contrast is so considerable that possibly there is some other contributing factor.

(vi) Incubation period

As early as 1815 Maton established that the incubation period of rubella was from 17 to 26 days and although most authors consider that it is usually 16 to 18 days, there is agreement with the range given by Maton

as extremes of 12 to 22 days (Dudgeon, 1967b) have been recorded.

In an experiment conducted by Anderson in 1949 rubella was transmitted to human volunteers by spraying the naso-pharyngeal region with infected throat washings; the rash appeared 13 to 20 days afterwards although lymph-adenopathy could be detected after 7 to 8 days. This work has been repeated with almost identical results (Green, Balsamo, Giles, Krugman, and Mirick, 1965).

d) Teratogenic effects of rubella

Up till 1941 no trace can be found in the literature of evidence that rubella contracted during the course of pregnancy is responsible for foetal malformation. In that year, however, an unusually large number of patients with congenital cataract appeared in Sydney, Australia. Dr. Norman M. Gregg, an ophthalmic surgeon noticed that this increased incidence of cataract occurred after a widespread epidemic of rubella the previous year. Gregg accordingly inquired closely into the health during their pregnancies of the mothers of the affected infants. He found that in the majority, infection with rubella had occurred early in pregnancy usually during the first or second months. After recovery the mothers had remained healthy till the birth of their babies. In his paper, "Congenital Cataract

following German Measles in the Mother" given to the Ophthalmological Society of Australia in 1941, Gregg recorded a series of 78 infants with congenital cataracts of whom 67 had a history of maternal rubella. The cataracts were often bilateral and 44 of the infants had also congenital defects of the heart of which patent ductus arteriosus was the most common. Microphthalmia was also frequently found and, in the retinae of several patients, irregular areas of pigmentation were present. The infants were often difficult to feed and appeared small and ill-nourished. Three years later Gregg described further patients in which the main defects were buphthalmos and mental deficiency in addition to cataracts (Gregg, 1944).

Gregg's findings were soon confirmed by Swan and his colleagues (Swan, Tostevin, Moore, Mayo and Black, 1943; Swan, Tostevin and Black, 1946) and other Australian workers (Carruthers, 1945; Patrick, 1948). Meanwhile, in many other parts of the world similar congenital defects after maternal rubella were reported - in the United Kingdom by Simpson (1944), Martin (1945, 1946) and by Clayton-Jones (1947); in the United States by Reese (1944), Rones (1944), Erickson (1944) and Wesselhoeft (1949); and in Switzerland by Franceschetti and Bourquin (1946).

In addition to the classical ocular, hearing and cardiac defects which became known as the "rubella syndrome" other congenital defects were later observed. Watson (1952) drew attention to the incidence of hepatosplenomegaly and Lundström (1952, 1962) to microcephaly. Berge, Brunnhage and Nilsson (1963) reported thrombocytopenia.

Thus, by 1963 a large amount of information had been gathered about congenital defects caused by maternal rubella. However, much more knowledge was soon to be gained as a widespread epidemic of rubella occurred in the United States of America between the end of 1963 and the summer of 1964 in which 1,800,000 persons developed the disease (Sever, Nelson, and Gilkeson, 1965). The additional features brought to light by this epidemic have been referred to as the "expanded rubella syndrome" or "acute disseminated rubella of the newborn".

A summary of the foetal defects now thought to be caused by infection with rubella during early pregnancy is shown in table 1.

Although Gregg was the first to note the teratogenic properties of the virus, descriptions of possible cases of congenital rubella can be found in earlier literature. For instance, James Wardrop in 1813 published an account of the history of James Mitchel, a boy born blind and deaf and

in 1892 Leuch described a person suffering from pulmonary stenosis associated with deafness. Although the authors do not refer to maternal rubella the lesions are in keeping with a diagnosis of congenital rubella.

Many attempts have been made since 1941 to assess the incidence of defects in infants born of mothers infected with rubella during the early months of pregnancy. Swan (1949) estimated from retrospective studies that 80 to 90 per cent of such infants were malformed. Later prospective investigations however showed a much lower proportion. Lundström (1952, 1962) found an incidence of 10.0 per cent in Sweden and in the United Kingdom during 1951-52, Manson, Logan and Loy (1960) found that 15.8 per cent of children whose mothers had rubella during the first twelve weeks of pregnancy had malformations when examined at two years of age compared with 2.3 per cent in a control group. In addition there was an increased risk of abortion and stillbirth.

Rubella contracted by the mother during the first month of pregnancy is more likely to produce a severe defect than in any of the subsequent months. Pitt and Keir (1965) studied 103 children whose mothers developed rubella during the first 16 weeks of their pregnancies; they reported major defects in 60 per cent of children after infection in

the first four weeks, in 33 per cent after infection from the fifth to twelfth week and in 5.7 per cent from the thirteenth to the sixteenth week of gestation.

Dudgeon (1967c) calculated that after maternal rubella during the first 16 weeks of pregnancy approximately 30 to 35 per cent of foetuses were affected when abortions and stillbirths were considered in addition to malformations.

e) Isolation and transmission of the rubella virus

Probably the first attempt to isolate the causative agent of rubella was made by Hess in 1914. He undertook a bacteriological examination of the blood of infants suffering from the disease and also inoculated rhesus monkeys with these specimens. In no case, however, was an organism isolated and apart from a rise in temperature noted in one of the monkeys nineteen days after inoculation none of them was otherwise affected.

In 1938 Hiro and Tasaka succeeded in transmitting the disease to children. They injected subcutaneously a filtrate ofnasopharyngeal washings collected from patients during the prodromal stage of the disease and up to 30 hours after the appearance of the rash, into 16 children without a previous history of rubella. A rash developed in four of the children and two had cervical lymphadenopathy without

any skin eruption.

Habel (1942) attempted to grow the virus on the chorio-allantois of chick embryos and although no lesions developed he was able to infect rhesus monkeys with this material after five sub-cultures. It has not been possible to confirm this work (Dudgeon, 1967b).

Twenty years were to elapse before the first successful isolations of the rubella virus were reported by workers in the United States. Weller and Neva working at the Harvard School of Tropical Public Health, Boston, isolated the agent from the blood and urine of four patients suffering from the disease by the inoculation of human skin-muscle tissue cultures and primary human amnion cultures; Parkman, Buescher and Artenstein at the Walter Reed Army Institute of Research, Washington, had similar success with material from the nasopharynx which they inoculated into primary African green monkey kidney cultures.

Within a short period of time isolation of the rubella virus became a standard procedure in many laboratories throughout the world.

f) Immunity to rubella

Shortly after the establishment of the laboratory diagnosis of rubella many advances were made in the understanding of the immunity to rubella. Studies in different

communities demonstrated that a large proportion of adult women possessed serum antibodies. Dudgeon (1965) noted that 84 per cent of women at a London obstetric hospital at the time of delivery, had antibody to rubella and Oxford (1966) found that the proportion was 76 per cent among pregnant women in the Sheffield area. In similar studies in Canada (Givan, Kozee, and Rhodes, 1965) 80 per cent of the women examined possessed antibody and in the United States (Sever, Schiff, Bell, Kapikian, Huebner, and Traub, 1965) 83 per cent.

Because of the large number of persons with antibody to rubella it is difficult, at least in Britain and America, to demonstrate differences between communities. In a report to the Public Health Laboratory Service Rubella Working Party in 1967 Hutchinson found that 83 per cent of serum specimens obtained from around Leicester contained rubella antibody compared with 94 per cent of those from Manchester. Although the difference is not very impressive it may indicate that a person from a congested city is more likely to have antibody to rubella.

The possession of serum antibody is related to immunity. This was strikingly shown by Green, Balsamo, Giles, Krugman and Mirick (1965) who attempted to infect children either by inoculation of serum obtained from patients

with rubella or by contact with such patients. Of 54 children who did not possess serum neutralising antibody in a dilution of 1 : 4, 46 (85 per cent) developed rubella, whereas all 37 children possessing antibody above this dilution were uniformly resistant to infection, regardless of the type of exposure. Moreover, susceptible individuals are usually devoid of antibody at the onset of infection and acquire it during convalescence.

The number of persons with serum antibodies found in the various communities is much greater than would be expected on the basis of a past history of clinical illness. The reason for this probably lies in the fact that asymptomatic infection is common. Horstmann, Riordan, Ohtawara and Niederman (1965) found the ratio of clinical to inapparent infection was approximately 1 to 1 but Buescher (1965) put the ratio as high as 1 to 6 in a study he undertook in army recruits. This important finding threw light on such problems as the birth of babies with deformities typical of the rubella syndrome but whose mothers had apparently not had rubella during pregnancy. Avery, Monif, Sever and Leikin (1965) reported seven such instances.

As a result of the large proportion of adult women who are immune, most newborn infants are protected at birth and for the first few months of life by maternal antibody.

By the sixth month of life this has usually disappeared and thereafter the incidence of antibody increases with age and the risk of exposure (Dudgeon, 1967b). Once acquired immunity appears to last for many years if not indefinitely. The study undertaken on the Pribilof Islands (Brody, Sever, McAlister, Schiff and Cutting, 1965) demonstrated that persons who had developed rubella 23 years previously were resistant to re-infection.

g) Prevention of rubella

Until the teratogenic properties of the rubella virus were recognised little effort was directed towards prevention of the disease. After 1941, however, energetic measures were taken in this direction.

Passive immunisation after exposure with either pooled plasma or immunoglobulin was the only available means of attempting to prevent the disease until the rubella virus was isolated. This advance, however, made possible the production of live vaccines.

(i) Passive immunisation

Numerous investigations have been carried out in various parts of the world to try and determine the protective efficacy of pooled plasma or immunoglobulin when given to rubella contacts. The first recorded account of pooled plasma being used in this way was given by Barenberg,

Levy, Greenstein, and Greenberg in 1942. They injected intramuscularly 30 ml. of pooled plasma to each of several children who were in a ward where there was intimate contact and repeated exposure to rubella. None of the inoculated children developed the disease. The authors did not state the number of children who took part nor did they have a control group. Although this investigation gave presumptive evidence of protection afforded by the pooled plasma the absence of a control group of uninoculated children made it difficult to know whether the disappearance of rubella was due to the administration of the plasma or to some other circumstance.

After this initial investigation a succession of studies to determine the value of immunoglobulin in contacts of rubella were made; the results indicated that this substance had also a protective effect (Korns, 1952; Anderson and McLorinan, 1953; Houser and Schalet, 1958; Grayston and Watten, 1959; Lundstrom, Thoren, and Blomquist, 1961).

More recently McDonald (1963) in a controlled investigation carried out at the Fountain Hospital, London, compared the incidence of the disease in a group of 94 children selected at random who were each given 250 mg immunoglobulin and an uninoculated group of 89 children

who acted as controls. None of the children had a previous history of the disease but all had been recently exposed to rubella before inoculation. Sixteen developed rubella including four children whose illnesses began within three days of the date of inoculation. Of the remainder only two (2.2 per cent) of the children given immunoglobulin developed the disease compared with 10 (11.4 per cent) of the controls, thus suggesting that immunoglobulin had some protective effect.

Another study demonstrating the prophylactic value of immunoglobulin was made in 1964 by Brody, Sever and Schiff in the Eskimo village of Barrow in Alaska. Rubella had been absent from the area during the previous 12 years and after its introduction the disease spread rapidly in children of 11 years of age and under. The epidemic began in December 1963 and ceased three months later after involving at least 69 of the 118 school children in the village. As the proportion of susceptible individuals was large a trial of immunoglobulin was carried out in the local school. After withdrawal of a blood sample 49 boys were given immunoglobulin (0.25 ml per pound bodyweight, rubella neutralising antibody titre 1/512, concentration not stated) and, on the assumption that it was better to allow girls to develop the disease to confer lasting immunity, they were left uninoculated to act

as a control group. Records were then kept of the occurrence of rubella in the two groups and also in 13 males who had not been given immunoglobulin. Approximately one month later a second blood sample was taken to detect rises in antibody titre. Before inoculation and for several days afterwards, cases occurred in both male and female children at approximately equal rates. As the epidemic progressed, however, the excess of cases among girls became very apparent. Of 56 girls who did not receive immunoglobulin 50 (89 per cent) developed rubella compared with only 9 (18 per cent) of the 49 boys who were inoculated. Of the affected boys five developed symptoms within six days of receiving immunoglobulin. Paired sera were available for 35 girls and 45 boys. Clinical and serological results agreed in most cases but 15 of the boys given immunoglobulin and who did not later have rubella developed antibody, whereas none of the girls had a rise in titre without having had a rash. Thus the clinical signs of rubella in these 15 boys were suppressed - probably by the immunoglobulin - although they were infected. Also of interest is the fact that although rubella virus was present in the community for at least a month after the date of inoculation there was no increase in cases towards the end of this period among persons who received immunoglobulin, which suggests that the protective

effect lasted for at least a month.

To assess the value of immunoglobulin in pregnant contacts of rubella in the United Kingdom McDonald (1963) analysed the case records of 12,927 women who received immunoglobulin between 1954 and 1961. The attack rate within 28 days of exposure in the home after a dose of 750 mg was 1.48 per cent and after 1500 mg, 1.13 per cent. A complementary study by the Royal College of General Practitioners (Watson and McDonald, 1963) noted attack rates for uninoculated women of between 17 and 44 years of age after a family exposure to rubella of 3.7 per cent, or of 5.5 per cent if women with a past history of rubella were excluded.

In a later report which analysed the results of inoculation of 30,764 pregnant contacts of rubella with immunoglobulin during the years 1956 - 62, McDonald and Peckham (1967) showed that only 1.95 per cent of family contacts and 0.48 per cent of non-household contacts subsequently developed the disease.

These studies all suggest that immunoglobulin protects rubella contacts, but unfortunately there is other evidence that protection is sometimes lacking.

Green, Balsamo, Giles, Krugman and Mirick (1965) at the Willowbrook State School, Staten Island, New York,

divided at random into two groups 70 children who were known to lack rubella antibody. To one group they gave immunoglobulin (0.12 - 0.20 ml/pound body weight, rubella neutralising antibody titre 1:32 to 1:64, concentration not stated) and the other group was left uninoculated. An attempt was then made to infect the children with rubella by various means. Twenty were given an intramuscular injection of serum containing rubella virus and in 19 others the serum was sprayed on to the pharynx. The remainder were exposed to the disease by contact with children who were already ill. The children were then followed up to detect clinical and serological evidence of infection. Of the 33 children who were given immunoglobulin 27 (82 per cent) developed rubella, 18 (55 per cent) having an illness with a rash; 34 (92 per cent) of the 37 uninoculated children became infected, 21 (55 per cent) with a rash. Thus there was no evidence that immunoglobulin had any prophylactic value when given to contacts of rubella.

In 1963 Schiff, Sever and Huebner suggested that the reason for the conflicting reports on the usefulness of immunoglobulin in the prevention of rubella was variation in the rubella antibody content of different batches. They found that there was an eight-fold difference in rubella neutralising antibody (256 to 2048) in 19 samples of

American immunoglobulin which they tested. Oxford (1966) noted a similar disparity in antibody titre between immunoglobulin of Dutch and British manufacture - the latter gave consistently higher rubella antibody titres.

McDonald (1963) considered that the time of administration of immunoglobulin in relation to the contact's exposure to the infection might also be a factor in determining protection: the attack rate in home contacts was less if the injection were delayed till the fifth day after exposure when the immunoglobulin might be more effective in control of the viraemic phase of the illness.

A further explanation of the disappointing results was given by Murphy and Reid (1967) who thought that as antibodies to immunoglobulin may be formed after previous therapy (Stiehm and Fudenberg, 1965) these may neutralise the effect of a future prophylactic dose.

It is clear that despite the number of studies confusion still exists on the value of immunoglobulin in preventing rubella. Although several of the investigations were uncontrolled and carried out before laboratory assistance was available, it is difficult to disregard the work of Brody, Sever and Schiff (1965) that gave convincing evidence of the value of immunoglobulin, nor the equally convincing study of Green and his colleagues (1965) who showed that it did not

prevent rubella. Whatever the explanation, none of the investigations that were controlled and complemented by serum antibody titrations, gave any information about the problem of protection of the rubella contact who is in early pregnancy; this of course is the vital issue.

(ii) Active immunisation

The isolation of the rubella virus, the better understanding of the causal relationship between maternal rubella and congenital abnormality, the uncertain benefit of passive immunisation against rubella and the births of large numbers of deformed infants after the rubella epidemic of 1963-64 in the United States gave new stimulus to efforts to develop some form of active immunisation against this disease. Formerly many authorities advocated the deliberate exposure of young girls to rubella in the hope that they would become infected and so develop natural immunity. This procedure has its dangers in that further spread of the disease cannot be readily controlled and, since many infections are mild or subclinical, the virus may reach women in early pregnancy (Lancet, 1966).

The first attempts to produce a live rubella virus vaccine were reported in 1966 (Parkman, Meyer, Kirschstein and Hopps, 1966; Meyer, Parkman, and Panos, 1966). The

strain of rubella virus that they used was attenuated by 77 passages in primary African green monkey kidney cell cultures. This strain, designated HPV-77 (high passage virus) produced an antibody response in 88 to 96 per cent of susceptible children (Cooper, Giles, and Krugman, 1968; Meyer, Parkman, Hobbins and Ennis, 1968; Lepow, Veronelli, Hostetler, and Robbins, 1968). Clinical rubella and viraemia were not noted in the children who received the vaccine and despite the fact that rubella virus could be recovered from the nasopharynx of 50 to 70 per cent of vaccinated children there was no spread of infection to susceptible contacts.

Although the vaccine did not evoke as great an antibody response as after the natural disease, the titre was sufficient to protect against subsequent infection. Five girls who had been vaccinated eight months to one year previously with HPV-77 were challenged intranasally with natural rubella virus (Meyer, Parkman, Hobbins and Ennis, 1968). None developed clinical or virological evidence of rubella. By contrast a control group of five unvaccinated susceptible girls all developed lymphadenopathy and three had a rash; on the twentyfirst day of illness rubella antibody was detected in all these unvaccinated girls. Thirty vaccinated persons in this study have now been

followed for a year and the rubella antibody induced by vaccination is still stable.

One potential hazard of a live rubella virus vaccine is of affecting the foetus if the mother were vaccinated during early pregnancy. Preliminary experimental work in pregnant monkeys (Parkman, Meyer, Hopps, and Kirschstein, 1967) showed that the attenuated virus does not cross the placental barrier. A more recent study in which pregnant women without antibody to rubella were vaccinated up to two weeks before undergoing a therapeutic abortion, failed to demonstrate that the virus had been transferred to the foetus (Furukawa, 1968). The numbers taking part in this investigation were small, however, and in view of the importance of determining the teratogenicity of the attenuated rubella virus this point requires further scrutiny.

h) Summary of the historical position to date

(i) After at least two centuries of confusion with measles and scarlet fever rubella is now established as a disease in its own right.

(ii) Although the clinical features are well recognised it may be difficult on physical examination to distinguish it from other diseases; it is therefore still misdiagnosed.

(iii) Rubella has a world-wide distribution and epidemics occur every seven to nine years; most cases have been noted in April and May.

(iv) The teratogenic effect of rubella contracted by the mother during the first four months of pregnancy has been confirmed by many studies.

(v) Rubella is caused by a virus, the isolation of which rapidly led to reliable diagnostic procedures.

(vi) Over 80 per cent of adult women in Europe and the United States have been found to possess rubella antibody.

(vii) Despite several investigations into the prophylactic effect of immunoglobulin, the value of this substance is still in doubt.

(viii) Studies with live-attenuated rubella vaccine have shown that it evokes an antibody response which is protective and that the attenuated virus does not spread to susceptible contacts.

P A R T II

FIELD STUDIES

CHAPTER 3

GENERAL PLAN

This chapter describes only in outline the different field studies that were undertaken and shows the way in which each contributed to the evaluation of immunoglobulin when given to pregnant rubella contacts.

Although the investigations were complementary it appeared logical to describe them separately with the results obtained. Thus each of the following three chapters gives an account of the procedures adopted for each study and reports on the findings. An account of the collection and handling of the data, laboratory methods and examples of protocols and record cards are given in the appendices.

In most of the investigations the virological examination of specimens was performed in the Public Health Laboratories at Bedford, Bristol, Carmarthen, Colindale, Coventry, Leeds, Liverpool, Manchester and Newcastle. As facilities were available at these laboratories the field studies were usually carried out in adjacent areas.

Because of the evidence that persons who have acquired antibody to rubella are resistant to reinfection with the rubella virus (Green, Balsamo, Giles, Krugman, and Mirick, 1965) a survey was undertaken to determine the

serum rubella antibody titres among women of childbearing age and so estimate the proportion at risk to infection. The relationship of the participant's antibody titre to the locality in which she lived, age, place of origin, history of previous illnesses diagnosed as rubella, number of previous pregnancies, miscarriages and stillbirths were noted.

It was believed that an initial investigation of this kind was necessary to form a foundation on which to base the subsequent studies.

To assess the prophylactic value of immunoglobulin given to women in the first four months of pregnancy because of recent contact with rubella it was considered important to determine three facts:

(i) the attack rate of rubella among pregnant contacts of the disease who had been given immunoglobulin;

(ii) the rubella antibody titres of the immunoglobulin used;

(iii) the attack rate of rubella among an adequate control group.

In England and Wales when a doctor is consulted by a patient in early pregnancy who has recently been in contact with a person suffering from rubella, he may obtain a supply of immunoglobulin from the nearest Public Health

Laboratory. Advantage was taken of this standard procedure to arrange for the collection of paired blood samples from pregnant rubella contacts, the first sample being taken immediately before administering the immunoglobulin and the second six weeks later. By comparing the antibody titres in the two samples it was possible to determine if infection had taken place. In addition the doctor was asked to take swabs from the throat and nose of the person who was suffering from rubella (the index case) so that the diagnosis could be confirmed by virus isolation.

To enable the prophylactic value of immunoglobulin to be assessed without the suspicion that any failure to protect might have been due to low rubella antibody titres in the batches used, samples taken from immunoglobulin issued from the manufacturers during the period of the investigation were titrated. Based on this information stocks of immunoglobulin of high rubella antibody titre were reserved for the study. In addition it was possible to observe the range of rubella antibody titres in the batches of immunoglobulin in current use.

In an ideal controlled investigation the pregnant rubella contacts would have been divided into two groups by random allocation and one group given immunoglobulin but

not the other. This procedure could not be followed for ethical reasons since there was previous evidence that immunoglobulin might protect the foetus. It was decided that the control group that came closest to the ideal comprised women of childbearing age who were in contact with rubella but who were not pregnant and so did not require protection. For this purpose doctors working in general practice were invited to send paired serum samples from non-pregnant rubella contacts and nose and throat swabs from the index cases. Thus the only differences between the inoculated and control groups were that one group consisted of pregnant women given immunoglobulin and the other of non-pregnant women not given immunoglobulin.

To determine the infectiousness of the rubella virus under different circumstances the following factors were considered:

(i) the attack rate of rubella after exposure to the disease in the home;

(ii) the attack rate after exposure outside the home;

(iii) the attack rate after exposure in a semi-residential community.

The risk of infection inside and outside the home among contacts was calculated from the information obtained

during the investigation to determine the prophylactic value of immunoglobulin.

An opportunity to study the risk among contacts of patients suffering from rubella in a semi-residential community arose when an outbreak occurred at a Police Training College. As with the previous studies paired blood samples were withdrawn from the contacts of the patients suffering from rubella and titrated for rubella antibody to determine the number who became infected.

These various investigations also gave information on the influence of the sex of the contact on the susceptibility to infection with the rubella virus. Similarly in female contacts it was possible to determine if pregnancy altered the susceptibility.

Because all the inquiries described in this thesis were based on laboratory as well as clinical findings it was possible to compare the attack rate of rubella determined on serological studies with that on clinical grounds and so estimate the frequency with which subclinical infections occurred.

Moreover, the incidence of subclinical infection among the women given immunoglobulin was compared with the incidence among the uninoculated control groups; in this way the question of whether immunoglobulin acts merely by

suppressing the clinical features of the disease rather than by preventing actual infection, was examined.

Since an attack of rubella results in the formation of antibody that is long lasting (Brody, Sever, McAlister, Schiff and Cutting, 1965) it may be inferred that the presence of rubella antibody reflects previous, but not necessarily recent, infection and that persons who lack antibody are unlikely to have had rubella in the past. The frequency of second attacks of rubella could therefore be assessed by noting the number of persons who already possessed antibody and had a further rise in titre after contact with rubella.

In summary, three linked investigations were designed to examine the various points raised in this thesis:

(i) a serological survey of adult females not recently in contact with rubella;

(ii) a comparison of the attack rate of rubella among women who had been given immunoglobulin after contact with the disease while in the first four months of pregnancy, with an uninoculated control group;

(iii) a study of the spread of rubella in a semi-residential community.

Because these investigations were conducted in various parts of the United Kingdom and involved many people - laboratory workers and doctors in general practice - it was necessary to have standard methods. These will be described in detail in the succeeding chapters but in general the following procedure was common to all the investigations.

Protocols describing the organisation of the studies and standard record cards were designed and distributed. Suitable specimen sets for taking throat and nose swabs and blood from patients were also sent to doctors. In addition control sera were issued to the participating laboratories to ensure that the serological results were uniform.

Until October 1967 the serum rubella antibody titres were determined by the neutralisation test. During the remainder of the investigation the haemagglutination-inhibition (H.A.I.) test was used exclusively as it was found to be more sensitive, less time-consuming, and gave comparable results (Field, Vandervelde, Thompson and Hutchinson, 1967). For this investigation serum neutralising titres greater than 1 : 4 and haemagglutination-inhibiting titres greater than 1 : 8 were regarded as indicative of immunity to rubella.

When completed the record cards were returned to the Epidemiological Research Laboratory, Colindale, London, where they were checked, coded and analysed.

CHAPTER 4

A SEROLOGICAL SURVEY TO RUBELLA OF ADULT FEMALES NOT RECENTLY IN CONTACT WITH THE DISEASE

Material and Methods

This survey to determine the proportion of women who possess antibody to rubella started in January 1966 and was completed in November 1967. Ten areas in England and Wales - Bedford, Bristol, Carmarthen, Coventry, Keighley, Leeds, Liverpool, London, Manchester and Newcastle - were involved so that geographical differences in serum antibody titres could be examined.

As it was intended to study women mainly of childbearing age, obstetricians in charge of ante-natal clinics were approached and arrangements made for the collection of about 2 ml. of venous blood at the time other samples routinely required during early pregnancy were obtained. Samples from older women were provided by other hospital departments.

A protocol describing the methods to be used and standard record cards (form 13) were distributed to the doctors taking part. The card recorded the following information: participant's name, address, nationality, age, possible history of rubella and date of infection, number of pregnancies, miscarriages and stillbirths and rubella antibody titre. Each card was stamped

with a number to identify the woman and the region in which she lived. Provision was made on the card for coding the information. Examples of the protocol and record card are shown in the appendix (pages xi, xii).

The record card was usually completed immediately before the blood sample was taken. Where this was not possible, the information was extracted from the patient's case sheet.

Blood samples were either dispatched directly to the local Public Health Laboratory or stored in a refrigerator at $+4^{o}C$ until transport was arranged.

Rubella antibody titrations were carried out by means of the serum neutralisation test (page v). Titres were entered on to the record cards which were then sent to the Epidemiological Research Laboratory for analysis.

Results

A total of 2,007 blood samples were collected from ten different areas of England and Wales. Table 2 shows the antibody titres of the sera according to area. Because of the proximity of Leeds and Keighley the results have been combined. Of the total, 184 (9 per cent) with titres less than 1 : 4 were regarded as susceptible and 1,779 (89 per cent) with titres of 1 : 8 or greater as immune due to past infection. In the small number 44 (2 per cent) with titres of 1:4 the results

have been regarded as equivocal as it is doubtful if a titre of this size is real evidence of past infection and probably represents non-specific viral inhibition in the neutralisation test.

The proportion of women susceptible to rubella varied in the different areas ranging from 20 per cent in the London area to 4 per cent in Leeds. This difference was statistically significant ($x^2 = 50.86$, $P < 0.001$). Coventry (5 per cent), Bedford (8 per cent) and Manchester (9 per cent) also had a low proportion of susceptible women. Taking all areas into consideration an average of 9 per cent of women of childbearing age were susceptible.

The rubella neutralising antibody titres found in the different age groups are shown in table 3 and figure 3. The age groups covered five-year periods from 15 to 45 years. The proportion susceptible fell from 16 per cent in those between 15 and 19 years of age to 1 per cent in those over 40 years. This difference was statistically significant ($x^2 = 28.2$, $P < 0.001$) but there was little change in the proportion susceptible in the age groups between 20 and 39 years.

Of the women investigated, 220 (11 per cent) came from countries outside the United Kingdom; West Indians contributed 35 per cent of this total, Indians and Pakistanis 20 per cent, Europeans 14 per cent and Africans 7 per cent (table 4). The

Europeans, Indian and Pakistani women had a similar proportion susceptible to rubella as British women. However, West Indian and African women had a higher proportion. Although the British group was much larger than the West Indian or African groups, the difference between the proportion susceptible was statistically significant ($x^2 = 25.12$, $P < 0.001$).

The history of a previous attack of rubella was examined in relation to the presence of neutralising antibody (table 5). Of the 545 participants who remembered having had an attack of rubella only 5 per cent lacked antibody compared with 12 per cent of the 1,100 who denied previous illness and 9 per cent of those who did not know. Again this difference was statistically significant ($x^2 = 20.23$, $P < 0.001$).

To examine whether women with large families would be more likely to possess immunity to rubella because of the increased risk of contact with an infected child, antibody titres, according to the parity of the woman are shown in table 6. Apart from the high proportion (29 per cent) of susceptible women among the small group who had never been pregnant, there was little difference in the distribution of antibody titres between the various groups. The difference did not attain statistical significance at the one per cent level ($x^2 = 7.25$, $0.02 > P < 0.05$).

Of the participants 411 (20 per cent) had a history of miscarriage or stillbirth. The distribution of the serum neutralising antibody titres according to the number of miscarriages or stillbirths reported is recorded in table 7. There was little variation in the titres between the different groups; women who had previously had a miscarriage or stillbirth were as likely to be susceptible to rubella as those without such a history.

CHAPTER 5

AN ASSESSMENT OF THE PROTECTIVE EFFECT OF IMMUNOGLOBULIN WHEN GIVEN TO CONTACTS OF RUBELLA WHO ARE WITHIN THE FIRST SIXTEEN WEEKS OF PREGNANCY

Materials and methods

The three linked studies considered necessary for the adequate evaluation of the prophylactic effect of immunoglobulin when given to pregnant rubella contacts are described in this chapter. The period of the investigation was from December, 1965 to December, 1968.

(i) Measurement of the rubella antibody titres of various batches of immunoglobulin in current use for rubella prophylaxis

Although most of the immunoglobulin issued in England and Wales is manufactured by the Lister Institute of Preventive Medicine, Elstree, Hertfordshire, it is distributed to practitioners by laboratories of the Public Health Laboratory Service which are supplied from a central stock held at the Epidemiological Research Laboratory. As the material usually spends some time at the Epidemiological Research Laboratory before distribution to the other laboratories the opportunity was taken to have samples of 15 batches issued by the Lister

Institute between December 1965 and July 1968 titrated for rubella antibody. Each batch was recently prepared and had at least two years till the date of expiry. The titrations were performed at the Virus Reference Laboratory, Colindale, using the rubella neutralisation test.

Five batches of immunoglobulin were reserved for the pregnant rubella contacts who participated in the investigation. Two of these batches had rubella neutralising titres of 1 : 320 (LKG 123 and LKG 131); the remainder had titres of 1 : 120 (LKG 127, LKG 138 and LKG 141).

(ii) Estimation of the attack rate of rubella among women who had been given immunoglobulin after contact with the disease while in the first four months of pregnancy

This enquiry was conducted in areas of England and Wales served by the following Public Health Laboratories: Bedford, Bristol, Carmarthen, Colindale, County Hall (London), Croydon, Coventry, Epsom, Exeter, Ipswich, Leeds, Liverpool, Newcastle, Northallerton, Nottingham, Oxford, Peterborough, Portsmouth, Salisbury, Sunderland, Swansea, Taunton and Winchester.

A protocol outlining the standard methods to be used and record cards (Pregnant Contact Record Card (1), Pregnant Contact Record Card (2), Index Case Record Card) were

distributed to all concerned. Examples of the protocol and record cards are shown in the appendix (pages xv, xix, xxi, xxiii). In addition, at least one visit was paid to participating laboratory directors so that difficulties could be discussed and a uniform policy arranged.

Each record card was stamped with a serial number to identify the patient, general practitioner and issuing laboratory.

To facilitate the transport of specimens from the general practitioner to the laboratory, special packs were designed (see page xvi) that contained the following items:-

1. One detailed instruction sheet (page xviii).
2. One McCartney bottle ($\frac{1}{2}$ fluid oz.) for collection of the blood sample.
3. Two sterile swabsticks (throat and nose).
4. Two bijoux bottles containing virus transport medium (page iv).
5. Two cardboard containers in which the McCartney and bijoux bottles were placed.
6. One leak-proof padded envelope.
7. One numbered Pregnant Contact Record Card (1).
8. One Index Case Record Card.
9. One pre-paid label for return of the specimens.

A stock of these items and a supply of immunoglobulin taken from the reserved batches were despatched to the various laboratory directors involved for issue to general practitioners.

The procedure at participating laboratories was as follows : when a practitioner requested immunoglobulin for a woman in the first four months of pregnancy who had been in recent contact with a patient suffering from rubella, he was told of the investigation and, if he agreed to take part, a pack including an ampoule containing 750 mg. immunoglobulin taken from the reserved batch, was sent to him.

On receipt of the pack, the doctor arranged to collect between 2 and 5 ml. of venous blood from the pregnant rubella contact, injected the immunoglobulin intramuscularly and entered the relevant clinical details on the Pregnant Contact Record Card (1).

When possible, and particularly when the source of rubella was a member of the same household, the doctor was also asked to take throat and nasal swabs from the index case. The swabs were immediately dipped into the virus transport medium contained in the two bijoux bottles. The doctor then completed the Index Case Record Card giving clinical details of the case of rubella. Because many of these patients were young children,

blood samples were not routinely collected from them.

Specimens were sent by the quickest available means (usually by post) to the laboratory designated to carry out the virological examinations.

If there were any delay in sending specimens the doctor was asked either to keep them at +4°C (i.e. usually in a domestic refrigerator) until suitable transport could be arranged or else to send the blood samples to the nearest Public Health Laboratory for separation of the serum and onward transmission to the appropriate centre.

Six weeks after the first blood sample was obtained, or sooner if it had been taken some time after the date of the pregnant woman's contact with the index case of rubella, a second pack containing a McCartney bottle, cardboard container, standard record card (Pregnant Contact Record Card (2)) and pre-paid label was sent to the doctor with a request for a further specimen of blood from his patient. The doctor was also asked to state whether or not the patient had developed the clinical signs of the disease. If the second specimen were not received at the laboratory a reminder was sent about a month later.

Until October, 1967, second blood samples were requested for all contacts who had given a first sample in order to determine the proportion of contacts suffering from second attacks

of rubella. By this time, however, it was considered that a sufficient number of paired specimens had been collected from those who were already immune. Thereafter the collection of second blood samples was restricted to those who were initially susceptible.

Also in October 1967 it was decided to increase the dose of immunoglobulin given to a home contact of rubella to determine if this reduced the attack rate. The initial dose was increased to 1,500 mg. In addition a further 1,500 mg. was sent to the contact's doctor if the first blood sample had a haemagglutination-inhibiting titre of 1 : 8 or less; the second dose was only sent if it could be given before the end of the incubation period. Thus ideally a susceptible home contact of rubella in early pregnancy was given four times the standard dose (750 mg.) of immunoglobulin during the latter period of the investigation.

When the antibody titre or the result of the examination of throat and nose swabs for rubella virus was known, a report was sent to the patient's doctor and a copy to the director of the laboratory that issued the immunoglobulin. The titres and isolation results were also entered on the record cards which were then sent to the Epidemiological Research Laboratory for checking, coding and analysis.

(iii) Estimation of the attack rate of rubella among non-pregnant women of childbearing age in contact with the disease but not given immunoglobulin

So that the results could be compared, this study was conducted during the same period of time as that to determine the attack rate of rubella in pregnant contacts given immunoglobulin.

A detailed protocol and record cards (Contact Record Card (1), Contact Record Card (2), and Index Case Record Card) were distributed to all the persons concerned in the project. The cards were similar in design to those used in the study concerned with the pregnant rubella contacts. Examples of the protocol and cards are shown in the appendix (pages xxviii to xxxiii). A pack was also produced resembling that already described (page 49) except that the ampoule of immunoglobulin was omitted.

Seventy-one general practitioners in England and Wales and three in Scotland were contacted, most with the help of the Epidemic Observation Unit of the Royal College of General Practitioners, and invited to participate. At least one personal visit was made to 31 of the doctors to explain the procedure. Detailed instructions were sent to the others by post.

Each practitioner who agreed to take part was sent twenty

sets of cards and packs. A further supply was despatched as required. The doctor was asked to take swabs from the throat and nose of any patient whom he suspected on clinical grounds to be suffering from rubella. He was also requested to obtain between 2 and 5 ml. of venous blood from any women of childbearing age (i.e. between 15 to 45 years) who were not pregnant but who were members of the same household as the index case. These specimens were sent to the appropriate laboratory where rubella virus isolation was attempted and antibody titrations performed. Sixty-two of the doctors found it easier to send the swabs and blood by post to the Virus Reference Laboratory at Colindale; the remainder delivered their specimens to the nearest participating laboratory. A report on the findings was returned to the doctor who sent the specimen.

If the rubella antibody titre in the first blood sample indicated that the contact was susceptible to rubella, a second sample was requested six weeks later to determine whether or not she had become infected. The doctor was asked to record any clinical signs of rubella that developed in the contact.

The rubella antibody titre of this second blood sample was also sent to the doctor from the laboratory and the results entered on the record cards.

To augment the number of women participating in this control group, each general practitioner who submitted specimens for the investigation involving pregnant rubella contacts (page 48) was requested by letter (page xxxvii) to provide suitable samples from non-pregnant contacts of rubella. A total of 379 doctors were contacted in this way.

Results

(i) Immunoglobulin

The rubella neutralising antibody titres of the 15 batches of immunoglobulin submitted for titration is shown in table 8. It was reported from the laboratory that difficulty was experienced in obtaining a definite rubella antibody end-point and because of this the titres of antibody are expressed as the highest dilution that substantially neutralised the virus.

Each batch contained rubella neutralising antibody, the titres ranging from 1 : 60 to 1 : 320. A titre of 1 : 120 was found in seven of the batches.

(ii) Inoculated group

Of the 1747 pregnant women who were given 750 mg. of immunoglobulin after contact with a patient suffering from rubella (diagnosed on clinical grounds and irrespective of viral isolation) 1483 (85 per cent) already possessed antibody to rubella. This figure is similar to that found in the serological

survey (table 2). Among the 264 women who lacked antibody and who were therefore susceptible to the disease 46 (17 per cent) developed serological evidence of infection (i.e. a four-fold or greater rise in antibody titre) in the second blood sample despite having had immunoglobulin. A clinical illness with a rash was present in 24 (52 per cent) of these women and the remainder had a subclinical infection (table 9 and figure 4).

The index case was a member of the same household as 28 of the susceptible pregnant contacts who developed rubella; of these women, 14 developed clinical illness and 14 had subclinical infections (table 10). Of the 18 patients who were infected outside the home, 10 developed a rash and eight were subclinically infected.

The clinical diagnosis was confirmed by the isolation of rubella virus from 126 index cases (table 11). The number of susceptible pregnant contacts of these cases was 25 (20 per cent) and 14 (56 per cent) of these developed rubella, 11 clinically and three subclinically.

In 75 instances where the index case was a member of the same household as the pregnant woman, 15 (20 per cent) of the women were still susceptible. Of these, 12 (80 per cent) subsequently became infected, nine clinically and three sub-

clinically (table 12, figure 5).

Contact outside the home with a proved case of rubella occurred in 51 instances and 10 of the women were susceptible. Of these only two became infected; both had illnesses with a rash. This difference in attack rate between susceptible pregnant women in contact within the home and those outside is significant at the 5 per cent level ($P < 0.05$).

The time interval between the date of onset of the rash in the index case, inoculation of the pregnant contact with immunoglobulin and development of the rash in the 14 susceptible contacts of proved rubella who became infected is shown in figure 6. Three of the 14 women were inoculated on the day the index case developed the rash and 7 others received globulin within three days of the rash appearing in the index case.

In the nine inoculated women who developed a rash after home contact with a confirmed case of rubella, the interval between the onset of the rash in the index case and the maternal rash ranged from 11 to 23 days (table 13). The interval was between 16 and 18 days in five of these nine women.

Among a further subgroup of 62 susceptible women in whom six cases of rubella developed (one clinical and five subclinical)virus isolation from the index case, although attempted, was unsuccessful. This may have been explained by the fact that

the throat and nose swabs were not collected until four to 10 days after the onset of illness.

The age distribution of the 1747 pregnant women who were given immunoglobulin after contact with rubella is shown in table 14; 1544 (88 per cent) were aged between 20 and 34 years of age. In the various age groups between 80 and 96 per cent of these women possessed antibody and, as found in the serological survey, older women were less likely to be susceptible.

Table 15 gives the results of the attack rate in the separate group of 57 susceptible women who were given two divided doses of immunoglobulin, each of 1500 mg, after home contact with a case of rubella. Of these women 14 (24 per cent) developed rubella, seven having an illness with a rash. However, six of the women were in contact with a patient from whom rubella virus was isolated; four of these six women developed rubella, all with a rash.

(iii) Control group

Five hundred and forty-three women from various parts of the United Kingdom were included in this aspect of the investigation and 493 (91 per cent) possessed antibody to rubella when examined (table 16, figure 7). All the index cases were members of the same household as the uninoculated contacts. Of the 50

women without antibody in contact with index cases diagnosed as rubella on clinical grounds 18 (36 per cent) developed the disease (16 clinically and two subclinically).

Rubella virus was isolated from 208 of the index cases but 182 (87 per cent) of their contacts already possessed antibody (table 17 figure 8). Of the 26 women who were susceptible 13 (50 per cent) subsequently developed rubella (11 clinically and two subclinically).

For these 11 women who developed a rash after contact with a confirmed case of rubella, the interval between the dates of onset of their rash is related to that in the index case in table 18. This interval ranged from 10 to 26 days; for six of the 11 women the interval was between 16 and 21 days.

Table 19 gives the age distribution of the 543 women in the control group; 372 (69 per cent) were aged between 20 and 34 years. The proportion of women who possessed antibody ranged from 88 to 97 per cent.

(iv) Index Cases

Information was provided for 874 index cases in the inoculated and control groups. The clinical features of these patients are shown in table 20. A rash was recorded in 97 per cent, lymphadenopathy in 82 per cent, fever in 61 per cent and arthritis in 3 per cent of patients.

Rubella virus was isolated from 334 (39 per cent) of the

848 patients with a rash (table 21). For the first three days after the onset of the rash the virus was isolated from between 49 and 55 per cent of the patients; thereafter the proportion of swabs that were positive dropped to between 25 and 30 per cent up to the sixth day after the onset of the rash. Only seldom was an isolation obtained after this time.

CHAPTER 6

A STUDY OF THE INFECTIOUSNESS OF THE RUBELLA VIRUS AND THE PROPORTION OF SECOND AND SUBCLINICAL INFECTIONS THAT IT PRODUCES

Material and Methods

(i) Infectiousness of rubella

In January 1968 an outbreak of rubella occurred at the Metropolitan Police Training College, Hendon, London. As the cadets spend at least six months at the Training College, working closely together in classrooms and sleeping in dormitories this seemed an ideal opportunity to study the spread of the disease in a community.

The initial cases were isolated at the Metropolitan Police Nursing Home, Denmark Hill, London. The nursing home was visited, the patients examined and clinical details recorded; swabs were taken from the nose and throat and blood samples obtained. The specimens were sent immediately to the Virus Reference Laboratory for attempted virus isolation and antibody titration. Repeat blood samples were collected from the same patients four weeks later.

After the first visit to the nursing home arrangements were made with the officer in charge of the training college for

the cadets in the same training section as those affected to be bled to determine the number who were still susceptible to rubella. The blood samples were rapidly screened by means of the haemagglutination-inhibition test and those cadets found to be susceptible were observed to determine if clinical disease occurred. A second blood sample was taken from susceptible cadets about six weeks after the first.

The clinical details, rubella antibody titrations and virus isolations were entered on record cards and the results analysed by hand.

(ii) Second attacks of rubella

Between December 1965 and October 1967 paired sera were collected from the women participating in the investigation to determine the prophylactic effect of immunoglobulin when given to pregnant rubella contacts, irrespective of whether antibody was present in the first sample.

To determine the proportion of second attacks of rubella a search was made for women who had pre-existing antibody but who developed a further attack of the illness.

As many index cases who have illnesses resembling rubella on clinical grounds are, in fact, suffering from diseases caused by other viruses, the study was confined to women in contact with an index case from whom rubella was isolated.

(iii) Subclinical attacks of rubella

The frequency with which inapparent rubella infections occurred and the question of whether immunoglobulin acts by suppressing the clinical features of the disease or by preventing infection, were investigated by means of the following procedure.

a) Examination of the records of women participating in the serological survey (page 42) to determine the proportion who possessed antibody to rubella, but who did not give a history of having had the clinical manifestations of the disease.

b) Noting the number of uninoculated contacts in the control group in the study to assess the value of immunoglobulin (page 53)and of those taking part in the police cadet study (page 61) who developed serological evidence of infection, but who did not have the signs and symptoms of rubella.

c) Determination of the number of subclinical attacks in pregnant rubella contacts given immunoglobulin.

Results

(i) Infectiousness of rubella

Between the 16th of January and the 20th of February, 1968, 17 cases of rubella occurred at the Metropolitan Police Training College; 13 of these developed during the last four days of this period (figure 9).

Swabs from the throat and nose and paired blood samples were obtained from these 13 cadets. Rubella virus was isolated from three of them and a four-fold rise in rubella antibody titre in one. The difficulty in confirming the clinical diagnoses in every cadet is explained by the fact that there was a delay of from two to five days between the onset of the rash and the date of the specimens being taken.

Rubella antibody titration of the blood samples taken from the remaining 224 cadets in the same training section as the cases revealed that 217 (97 per cent) already possessed antibody.

The cadets were recruited from many parts of the British Isles (table 22 and figure 10) although 162 (72 per cent) came from London and the Home Counties. Because of the small number of persons who were susceptible it was not possible to demonstrate if there was any geographical influence on susceptibility.

Of the seven cadets found to lack antibody, all developed serological evidence of rubella and six had an illness with a rash (table 23). Details of these cases are given in table 24.

(ii) Second attacks of rubella

Between December, 1965 and October, 1967 there were 106 women (84 pregnant and 22 not pregnant) taking part in the study of the assessment of immunoglobulin (page 47) who possessed

rubella neutralising antibody greater than 1 : 4 or haemagglutination inhibiting antibody greater than 1 : 8 and who were in contact with confirmed index cases of rubella (table 25).

Of these women only one pregnant mother developed a possible second attack of rubella. She was in contact with her son who had typical rubella with a rash, enlargement of the posterior cervical lymph nodes and fever. Rubella virus was grown from a nasal swab taken from the son on the day following the onset of the rash. As the mother was 10 weeks' pregnant at the time of her son's illness a blood sample was taken and she was given 750 mg. immunoglobulin intramuscularly. The rubella neutralising antibody titre of this batch of immunoglobulin was 1 : 320. There was a delay of only one day between the onset of the son's rash and the date of the blood sample and inoculation of the mother. Fifteen days after the onset of the son's rash, however, the mother developed a rubelliform rash and rubella virus was isolated from a throat swab taken from her at the time of the rash. A second specimen of her blood obtained four weeks later showed that the neutralising antibody titre was almost unchanged (1 : 24 in the first specimen and 1 : 32 in the second). This result probably lies within the limits of the error of the test as the titres

obtained by the complement-fixation test remained stationary at 1 : 16 as did those demonstrated by the haemagglutination-inhibition test at 1 : 256 but the isolation of rubella virus from the mother suggests that this was indeed a second attack. In none of the 106 women studied did a four-fold increase in the antibody titre occur although 14 showed an equivocal variation.

(iii) Subclinical attacks of rubella

The frequency of subclinical attacks was estimated from the following evidence.

Of 2,007 women examined in the rubella antibody survey (page 42) 1,100 (55 per cent) stated that they had not experienced a previous clinical attack of rubella (table 5). Only 157 (15 per cent) of these 1,100 women had rubella neutralising antibody of 1 : 4 or less. Thus the remaining 943 women either had had a subclinical attack of rubella or their memory of the illness was at fault. Although it is likely that many would have forgotten having rubella since infections probably occurred in childhood, it seems reasonable to suppose that in a proportion the discrepancy between past history and serological findings was due to subclinical infection.

Better evidence was found in the study to determine the attack rate of rubella in the control group of non-pregnant rubella contacts (page 53) Of the 543 contacts, 18 developed

rubella confirmed serologically. Only two (11 per cent) developed a four-fold or greater rise in rubella antibody titre but did not have any of the clinical features of rubella (table 16). Also in the police cadet study only one patient of the seven who developed rubella, had a subclinical infection (table 23).

Of the 46 pregnant rubella contacts given immunoglobulin and who later developed rubella, 22 had a subclinical infection (table 9) whereas only two of the 18 cases in the control group were infected without having a rash (table 16). This difference is statistically significant ($x^2 = 5.9$, $P < 0.02, > 0.01$).

CHAPTER 7

DISCUSSION

Rubella is a particularly difficult disease to study without laboratory help. The clinical illness is usually vague and fleeting and liable to be confused with other conditions. The isolation of the rubella virus in 1962 and the introduction of the laboratory diagnosis was therefore a very important development especially in view of the teratogenic properties of the virus.

Before this major advance, it had been definitely established that rubella is infectious, produces signs and symptoms which are usually mild and frequently causes foetal abnormality when contracted by women in early pregnancy. These facts are still undisputed.

However, there was also considerable evidence that immunoglobulin exerted a protective effect when given to pregnant rubella contacts. This evidence was obtained by observing the proportion of women given immunoglobulin after contact with rubella who did not develop the clinical features of the disease. The early workers were neither able to confirm the diagnosis, nor to determine if the person were already immune to rubella before contact. Because the presence of antibody has been shown to

protect against infection with rubella (Green, Balsamo, Giles, Krugman and Mirick, 1965) immunoglobulin given to contacts of the disease who already possessed antibody was almost bound to be"successful". To adequately assess the protective effect afforded by immunoglobulin given to pregnant women after contact with rubella it is necessary to confine the investigation to susceptible women in contact with confirmed cases of the disease. A suitable control group consisting of uninoculated women in similar circumstances is also required. Therefore a reliable assessment of immunoglobulin could not take place before laboratory help was available because information on the susceptibility of the contact and the confirmation of the diagnosis could not be obtained by other means.

By 1965, however, isolation of the rubella virus and determination of the rubella antibody titre was sufficiently well established to regard the laboratory diagnosis of infection as reliable. It was therefore possible to enquire into these problems on a much sounder basis than had hitherto been possible.

Immunity to rubella

The proportion of immune persons in the populations involved in the various aspects of this investigation was found to be consistently high - 89 per cent in the survey of adult women not in recent contact with rubella (table 2); 85 per cent of pregnant contacts of rubella (table 9); 91 per cent of the

control group of non-pregnant women (table 16) and 97 per cent of the cadets involved in the training school outbreak (table 23). These high proportions of persons already immune are similar to others published which range from 76 to 84 per cent (Dudgeon, 1965; Givan, Kozee, and Rhodes, 1965; Sever, Schiff, Bell, Kapikian, Huebner, and Traub, 1965; Oxford, 1966).

Some degree of variation in the percentage of women immune to rubella was noted between the populations in the various regions studied. The area with the largest percentage of immune women was that drawn from around Leeds and Keighley. Here 96 per cent of the women tested possessed antibody to rubella. It is surprising that the other northern cities taking part - Newcastle, Manchester and Liverpool - did not have similar results but the difference is almost certainly due to a true geographical variation and not a difference in laboratory technique in view of the standardisation of procedures that took place at the beginning of the investigation (see appendix, page v).

Because most of the blood samples obtained for this survey came from women attending ante-natal clinics in various towns and cities, the proportion of immune persons is possibly larger than if rural areas were studied. This may not in fact be the case however, as Field (1967) noted that 95 per cent of the inhabitants of a rural village in Wales possessed rubella

antibody at the time of examination.

As might be expected because of the persistence of rubella antibody after infection (Brody, Sever, McAlister, Schiff, and Cutting, 1965) there were more susceptible persons in the younger than in the older age groups. The proportion of those susceptible fell from 16 per cent in women under 20 years old to less than 10 per cent in older persons. Only 1 per cent of those over 40 years of age were susceptible (table 3). Thus the risk of developing the disease after contact is higher in young persons although even in those under 20, the majority were already immune.

Eleven per cent of those studied in the survey came from outside the United Kingdom (table 4). The proportion of European, Indian and Pakistani women who were immune was similar to that found in British women. The West Indian and African communities on the other hand had a lower proportion of immune women. This difference was statistically significant and it is possible that there is a racial susceptibility to rubella. The number in these groups however, was too small to come to any firm conclusion.

Before it was possible to determine serologically whether a person possessed rubella antibody or not, reliance in detecting immunity had to be placed on the memory of a previous attack of the disease. On comparing the rubella antibody possessed by a patient with her memory of a previous clinical attack (table 5)

this method of determining immunity is shown to be highly unreliable. Fortunately the error is usually to assume that a person is not immune when in fact she does possess antibody. Only 5 per cent of the women who stated that they had contracted rubella in the past did not have rubella antibody. Of those who denied a previous attack of rubella, however, only 12 per cent lacked antibody, a pointer to the possible high rate of sub-clinical illness.

A susceptible woman is at greater risk of contracting rubella inside her home, usually from one of her children, than she is from contact outside (table 12). It is, therefore, not surprising that the proportion of women with antibody was higher in those with children (table 6), probably because the usual nurse of a child with rubella is its mother. It could also be argued that women without a family may have a different social background and live in a less crowded neighbourhood that those with several children; these women may not therefore have had the same opportunity to contract the disease.

Apart from foetal malformations about five per cent of mothers who develop rubella during the first 12 weeks of pregnancy have miscarriages or stillbirths as compared with about two per cent in a control group of healthy mothers (Manson, Logan and Loy, 1960). Nineteen per cent of the women taking part in the rubella antibody survey had one or more miscarriages

or stillbirths prior to the investigation (table 7). The proportion of these women who possessed antibody to rubella, however, was only slightly higher than the proportion in those who had a better obstetric history. This failure to demonstrate a difference between the groups is probably due to the fact that the total number of women in the survey who were susceptible was small.

Protective effect of immunoglobulin

Because the majority of persons in the United Kingdom possess antibody to rubella and as immunoglobulin is prepared from pools of plasma obtained from adult donors, it follows that British immunoglobulin is likely to contain rubella antibody. The results given in table 8 verify that all the batches examined contained antibody so that it is unlikely the manufacturing process affects the antibody titre to any large extent.

Immunoglobulin has been used in the United Kingdom since 1954 for the protection of women in contact with rubella during early pregnancy. Various reports on its prophylactic value have been given (McDonald, 1963; Brody, Sever, and Schiff, 1965; Green, Balsamo, Giles, Krugman, and Mirick, 1965). As recently as 1967, McDonald and Peckham, in an analysis of 30,764 pregnancies complicated by contact with rubella, suggested that a degree of protection was given by immunoglobulin. The field

work of the investigation, however, was completed in 1962 and because of this, virological studies were not carried out. These workers therefore, were unable to take into account the large number of women who were already immune before immunoglobulin was given, those developing a subclinical attack, and those in contact with diseases other than rubella.

To avoid these sources of error, every woman who participated in the present studies was screened for the presence of rubella antibody. When only susceptible women were considered, irrespective of whether the illness in the index case was always true rubella, 17 per cent of the pregnant rubella contacts given the standard dose of 750 mg. immunoglobulin developed serologically proved rubella (table 9). With the further restriction of confirmation of rubella in the index case by virus isolation, 56 per cent of susceptible pregnant contacts became infected (table 11). It was also noted that of the 15 susceptible women in contact with a proved case of rubella who was also a member of the same household, 12 (80 per cent) subsequently developed the disease even when they were inoculated early in the incubation period (table 12, figure 6). This illustrates that the extremely high attack rate among susceptible pregnant contacts can only be revealed by considering separately those women in contact with a proved case of rubella in the home where there is a high likelihood of spread of infection.

If immune contacts are not excluded from the analysis and if no account is taken of the number of contacts who develop subclinical attacks of rubella then the true position is obscured. Thus only 24 pregnant women out of a total of 1747 in contact with rubella actually developed a clinical illness with a rash, an attack rate of only 1.4 per cent (table 9). This is very similar to the attack rate of 1.2 per cent found by McDonald and Peckham (1967) and on this basis one could perhaps have thought that immunoglobulin exerted a protective effect.

In addition to the consideration of the attack rate of rubella among contacts given immunoglobulin, it was particularly important to have a control group in this investigation. The numerous factors already mentioned that determine the proportion of women who become infected (e.g. percentage of women with pre-existing antibody, closeness of contact with the index case, and accuracy of diagnosis of the illness in the index case) may influence the result quite apart from the effect of immunoglobulin.

The control group used was not ideal - they were not pregnant and although all of childbearing age, the women were slightly older (tables 14 and 19). Despite these limitations the comparison of the attack rates between the groups did not suggest that immunoglobulin at the dosage used was giving any protection to the pregnant contacts.

Two factors may have been present to give such poor results - size of the dose and time of administration.

a) Dose

Brody, Sever, and Schiff (1965) demonstrated that immunoglobulin was protective when given to rubella contacts. As their investigation included antibody studies, subclinical attacks were unlikely to have been missed and the number of immune persons in the population was known. Also there was an adequate control group. Brody however, gave a much higher dose of immunoglobulin than that normally used in the United Kingdom. He injected an amount dependent on the weight of the individual - 0.25 ml per pound body weight. Although he did not state the concentration of immunoglobulin it was presumably that normally issued to American contacts, i.e. 16 per cent. This dose, if given to a woman weighing 9 stone, would amount to approximately 4,500 mg. - six times larger than the standard British dose.

McDonald (1963) found that the attack rate in pregnant rubella contacts varied between 1.48 per cent after a dose of 750 mg. immunoglobulin and 1.13 per cent after 1,500 mg. The difference, however, was not statistically significant ($P = 0.1$).

Additional evidence that a larger dose of immunoglobulin may give better results was put forward by members of the Public Health Laboratory Service Working Party on Rubella (1968a) who showed that circulating rubella antibody was not observed in

susceptible women after a 750 mg. injection of immunoglobulin, whereas low antibody titres were obtained in those given 1,500 mg; a divided dose of 3,000 mg. resulted in detectable antibody for 12 weeks.

Despite these encouraging reports, 14 (24 per cent) of 57 susceptible pregnant home contacts of rubella in the present study developed the disease even although they had received 3,000 mg. of immunoglobulin (table 15). When the analysis is limited to the six susceptible women in contact with confirmed cases of rubella, four developed rubella.

b) Time of administration

Usually the first warning a pregnant mother gets of the presence of rubella in a person with whom she has been in contact, is the appearance of a rash. However, most cases are probably infectious before this as Green, Balsamo, Giles, Krugman, and Mirick (1965) isolated the virus from the naso-pharyngeal secretions of cases up to seven days before the rash appeared. Thus, even if immunoglobulin is given to the mother promptly after the appearance of the rash in the index case, she herself may well be incubating the disease by this time.

In this current investigation the interval between the date of onset of the rash in the index case and that in the contact was under 16 days in three of the inoculated women (table 13) and four of the controls (table 18) who developed

rubella. These intervals suggest that the contact became infected before the index case developed the rash.

A report by the Public Health Laboratory Service (1968b) on the prophylaxis of infectious hepatitis demonstrated the advantage of giving immunoglobulin as early in the incubation period as possible. This may also apply in the prevention of rubella. However, Green and his colleagues (1965) in their series of experiments, varied the time of administration of immunoglobulin from between five days before exposure to rubella to 24 hours after exposure. No protective effect was noted with any of these regimes, and the time of administration of immunoglobulin was considerably earlier than can be given in practice to a pregnant rubella contact.

A further difficulty that arises when immunoglobulin is used prophylactically in rubella is that it may suppress the clinical features of the disease but not prevent infection. The proportion of subclinical infections in inoculated women who developed rubella was 48 per cent (table 9) whereas only 11 per cent of the infected controls had a subclinical illness (table 16). This effect of immunoglobulin has already been demonstrated by Ward and Krugman (1962) in the prophylaxis of infectious hepatitis. They showed that the number of clinical cases of infectious hepatitis among contacts was substantially reduced after inoculation but the attack rate as measured by

abnormal liver function tests was unaffected.

Although Horstmann (1968) suggested that in subclinical infections the duration of viraemia and the virus dose to the foetus is probably decreased, she admitted that babies with the rubella syndrome may be born to mothers who do not give a history of rash or other clinical manifestation of rubella. The degree of risk to the foetus from maternal subclinical infection requires further study but this type of infection is also dangerous for two reasons: first, the mother may be lulled into a false sense of security if serological studies are not performed after immunoglobulin has been given and the infection is not detected; second, if she has become infected and is unaware of it, she may spread the virus to other individuals - especially other pregnant women with whom she comes into contact, e.g. at an ante-natal clinic. Evidence that subclinical cases are likely to be infectious has been given by several workers experimenting with rubella vaccine (Cooper, Giles and Krugman, 1968; Lepow, Veronelli, Hostetler, and Robbins, 1968; Meyer, Parkman, Hobbins and Ennis, 1968). They found that susceptible persons given the vaccine, underwent serological conversion and excreted the virus but did not develop the clinical features of the disease.

A susceptible pregnant woman in contact with a case of confirmed rubella in her own home can expect therefore little

protection from the dose of immunoglobulin currently offered. It is unlikely that this situation can be greatly improved until rubella vaccines become generally available. The larger dose of 3,000 mg. of immunoglobulin investigated in the present study represents a large (20 ml) and painful injection. Moreover, immunoglobulin is in short supply in the United Kingdom, a considerable quantity having to be imported from French, Swiss and Dutch sources. It is also expensive - one 750 mg. ampoule costs £1. 7. 0. (Department of Health and Social Security, 1968). To give the dose that Brody found to be protective would cost £8. 2. 0. for each contact and as the volume of this dose of immunoglobulin would amount to over 30 ml. it would require several injections.

If massive doses were considered the demand could be eased if immunoglobulin were given only to those found to be susceptible. Over 80 per cent of women in the United Kingdom already possess rubella antibody and they are unlikely to require further protection in view of the small number of second attacks of the disease that occur (page 64). The difficulty, however, is separating immune from the susceptible contacts early enough so that the latter may be injected with a larger dose. The haemagglutination-inhibition and fluorescent antibody tests are likely to be some help in this direction as with these, it is possible to get a result within two days. However, by this time

the patient may be well into the incubation period as there is delay - avoidable and unavoidable - at every stage: from the time the pregnant mother notices the patient's rash until she goes to her general practitioner; in sending a blood sample to the appropriate laboratory for antibody titration; in getting the result back to the doctor and sending him a supply of immunoglobulin; until the patient returns to the doctor for the immunoglobulin which may require, if a large dose is given, several injections over a number of days.

A further difficulty is that some of the cases in contact with pregnant mothers may well be subclinical and therefore go undetected, as virological studies would normally only be carried out if the index case happened to be pregnant.

Probably the only satisfactory way to ensure that susceptible women in the first four months of pregnancy get a large amount of immunoglobulin quickly enough is to determine their immunity before they are in contact with the disease. This would require mass screening of women to assess their rubella antibody status irrespective of their previous history of clinical illness. Those found to be susceptible could then present themselves for inoculation as soon after contact as possible and not have to wait for the result of an antibody titration. Brody, Sever and Schiff (1965) considered that for maximum protection it might be wise to administer immunoglobulin

to women considered at risk (e.g. nurses and teachers) even before a known exposure to rubella has occurred.

As already discussed a pregnant woman is much more at risk from a member of her own family - usually one of her children - than from an outside contact. Possibly therefore the best time to test her serum for rubella antibody is when she is pregnant for the first time. This is because it is likely to be this child who may at a later date infect her with rubella when she is pregnant again. Apart from this, the timing is convenient as she will usually have a blood sample taken during this period in any case for such things as blood grouping and haemoglobulin estimation.

The cost of a screening procedure is considerable. An estimate for staffing and equipping one laboratory has been made by Tobin (1966). He has calculated the initial price to be in the region of £5,000 but such a laboratory could serve a large area of the country and it would not be necessary to renew the equipment yearly. In comparison, immunoglobulin costs the United Kingdom annually about £25,000 when 750 mg. is given to pregnant rubella contacts on request. If Brody's dosage level is adopted the cost would be £150,000 annually and at least 80 per cent of this would be wasted because of the proportion of immune women.

Thus if the use of immunoglobulin is to continue there

is little doubt that by far the most economical method of attempting to protect pregnant women from the rubella virus until a rubella vaccine is available, is to separate out those who are susceptible and give them very large doses of immunoglobulin immediately after or preferably just before they are in contact with a patient suffering from the disease. Apart from economic grounds, this method is more likely to be successful in protecting the foetus.

Another approach to the problem is to carry out serological tests for rubella antibodies before pregnancy and offer a therapeutic abortion to those found to be susceptible and who subsequently develop rubella during the first four months of pregnancy (Ross, 1968). Horstmann (1968) supports this view and suggests that immunoglobulin is reserved for women who, because of religious and other grounds, refuse to have the pregnancy terminated. Certainly on the present evidence a susceptible pregnant woman in contact with rubella cannot be reassured after an injection of immunoglobulin.

Attack rate of rubella

The attack rate in susceptible women in close contact with confirmed rubella was 50 per cent in the uninoculated controls (table 17) and 80 per cent in those who were pregnant and given immunoglobulin (table 12). Although not statistically

significant ($x^2 = 0.8$, $P < 0.2, > 0.1$), this finding suggests that susceptibility to rubella may be altered by pregnancy. An increased susceptibility to paralytic poliomyelitis in pregnant women has been noted previously (Siegel and Greenberg, 1955); presumably this is due to hormonal changes. There is an increased level of 17 hydroxycorticosteroids in the blood during pregnancy (Gemzell, 1953) and Venning (1946) also observed that the urinary excretion of corticosteroids is increased during the first trimester of pregnancy. Steroid hormones are known to inhibit initiation of the antibody response (British Medical Journal, 1966) and persons taking corticosteroids are especially prone to severe attacks of other viral diseases such as chickenpox (Haggerty and Eley, 1956; Johnson and Nelson, 1960).

The attack rate among the police cadets was 100 per cent. Although the number of susceptible persons was small in this investigation it would appear that the lower attack rates previously noted among males as compared with females (Royal College of General Practitioners, 1963) was probably due to lack of exposure rather than a decreased susceptibility to rubella.

Second attacks of rubella

Only one woman in this investigation developed a second attack of rubella although for nearly two years paired sera

were taken from as many rubella contacts as possible, immune and susceptible, in search of a rise in titre.

It would appear therefore that in the majority of cases a previous attack of rubella confers protection for at least a considerable period of time. This finding has been previously reported several times (Sever, Schiff, and Traub, 1962; Buescher, 1965; Green, Balsamo, Giles, Krugman, and Mirick, 1965; Horstmann, Riordan, Ohtawara, and Niederman,1965; Plotkin, Cornfeld, and Ingalls, 1965; Meyer, Parkman, and Panos, 1966; Meyer and Parkman, 1967; Meyer, Parkman, Panos, Stewart, Hobbins, and Ennis, 1967). Although increases in titres of pre-existing antibodies have been reported in persons exposed to a subsequent infection of measles, or when given measles vaccine (Krugman, Giles, Friedman, and Stone, 1965; Dolgin, Levine, Markham, Cabasso, Weichsel, Ruegsegger, and Cox, 1960) these "booster" type antibody responses have not been noted in immune persons after rubella exposure. This observation is confirmed in this study; only 14 two-fold increases in titre occurred in 106 immune individuals who were in contact with virologically proved rubella (table 25). This increase in titre was considered to be within the limits of laboratory error.

It is unfortunate that one second attack did occur as otherwise one could give unequivocal assurance to the immune pregnant rubella contact. The risk, however, is at least less

than one per cent.

Clinical findings and isolation of the rubella virus

The analysis of the clinical features of the index cases of rubella (table 20) supports the findings of Young and Ramsay (1963) that a rash was an almost constant feature of the disease and that posterior cervical lymphadenopathy was very common. The low incidence (3 per cent) of arthritis was probably due to the fact that nearly all the index cases were children and it has been previously noted that this complication is more common in adults (Fry, Dillane and Fry, 1962).

The virus was isolated from over half the patients in the first three days after the onset of the rash (table 21). These patients were more infectious at this time than later as the isolation rate dropped to between 25 and 30 per cent during the next three days; thereafter the virus was rarely isolated. This confirms the experimental work of Green and his colleagues (1965) and emphasizes the importance of taking swabs as near to the date of onset of the rash as possible in order to obtain a positive isolation.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

That the rubella virus must infect a large proportion of people both clinically and subclinically at an early age is shown by the fact that between 80 and 97 per cent of the individuals who took part in these studies already possessed antibody to rubella. As only one second attack of rubella was noted it must be assumed that the majority of persons who possess antibody to rubella are protected from a further attack. Only the small proportion who do not possess antibody are at risk and therefore require protection.

By protecting the mother from rubella during the first four months of pregnancy it is hoped that foetal malformation is also prevented. Clearly passive immunisation with the present dose of immunoglobulin is of little value. Possibly a larger dose would be more satisfactory but this, in its turn, brings about such difficulties as the administration of large volumes of fluid and a greatly increased demand for a commodity that is already in short supply and is expensive.

During the past three years intensive research has taken place into the production of a live-attenuated vaccine

against rubella. This had produced encouraging results and there is every likelihood that this vaccine will effectively eliminate the risk of infection to susceptible pregnant women. The administration of immunoglobulin with all its attendant difficulties will therefore become unnecessary. Until a vaccine comes into general use, however, it is likely that immunoglobulin will continue to be given to susceptible women in contact with rubella during the first four months of pregnancy.

Recommendations

1. Every woman of childbearing age should have their serum titrated for rubella antibody.
2. If susceptible, they should be advised that, if they become pregnant, they should avoid contact with patients suffering from rubella. This is especially important in the case of schoolteachers, nurses in paediatric wards where they may come into contact with babies suffering from congenital rubella and those working in infectious diseases hospitals.
3. Should a suceptible woman who is within the first four months of pregnancy anticipate that she is liable to come into unavoidable contact with rubella (e.g. during an epidemic) she should be given large doses of immunoglobulin (at least 4,500 mg.) that have been taken from a batch of high rubella antibody titre. If she is in contact with

a patient suffering from rubella a serum sample should be obtained as soon after contact as possible and again five to six weeks later to determine whether she has become infected. If infection occurs therapeutic abortion may be offered.

4. Because of the shortage of immunoglobulin, priority for large doses should be given to:
   (a) contacts who are in very early pregnancy because of the greater risk of major foetal malformation;
   (b) pregnant household contacts because of the greater likelihood of infection than when the index case is outside the home.
5. Alternatively, if a susceptible woman is in contact with rubella, immunoglobulin may be withheld in view of the serious doubts about its efficacy, and therapeutic abortion offered to those who become infected.
6. The haemagglutination-inhibition test should replace the neutralisation test for the serological diagnosis of rubella since a result is obtained much more quickly.
7. As active immunisation with a live-attenuated rubella vaccine appears to be a more promising proposition in the prevention of rubella than the administration of immunoglobulin, every effort should be made to bring a satisfactory vaccine into general use as soon as possible.

CHAPTER 9

SUMMARY

A series of epidemiological studies has been carried out among women in several areas of the United Kingdom and among cadets of the Metropolitan Police Force with the object of investigating various aspects of the natural history and prevention of rubella.

Immunity to rubella

1. The majority of persons investigated already possessed antibody to rubella by the time they reached adult life. The proportion immune varied in the different geographical areas studied and ranged from 96 per cent of the women from Leeds and Keighley to 80 per cent of those from London. Taking all the regions into consideration 91 per cent of the women examined were immune.

2. Immunity to rubella varied to some extent with age. Of those less than 20 years, 84 per cent were immune compared with over 90 per cent in older women.

3. Among the immigrant population living in the United Kingdom, the African and West Indian populations appeared to have a smaller proportion of persons who possessed rubella antibody.

4. The reported history of an attack of rubella did not agree with the patient's immunity to rubella.

5. Women without children were more likely to be susceptible to rubella than women with families.

6. A large proportion (97 per cent) of the young adult males investigated also possessed antibody to rubella.

Immunoglobulin

7. Ampoules from fifteen batches of British immunoglobulin were tested for neutralising antibody to rubella. All contained antibody, the titres ranging from 1 : 60 to 1 : 320.

Attack rate in rubella contacts given immunoglobulin

8. (a) When no account was taken of the immunity of pregnant women in contact with cases diagnosed clinically as having rubella, only 1.4 per cent developed an illness with a rash after having had an inoculation of 750 mg. immunoglobulin. (b) When virological studies were performed, however, and the analysis restricted to susceptible pregnant women who were in home contact with a case of rubella proved by isolation of the virus, then 80 per cent became infected either clinically or subclinically despite having had 750 mg. immunoglobulin.

9. Even when the dose of immunoglobulin was increased to

3,000 mg., four of six susceptible women developed the disease after contact with a confirmed case of rubella.

10. The risk of contracting rubella was much less if the index case was not a member of the same household. The attack rate for inoculated, susceptible pregnant women in contact outside the home with a confirmed case of rubella was a quarter of the rate found for home contacts.

Attack rate in uninoculated contacts of rubella

11. Fifty per cent of uninoculated susceptible women of childbearing age developed rubella after home contact with a virologically proved case. As 80 per cent of pregnant women became infected, pregnancy may increase susceptibility.

12. In the Hendon Police College - a residential community - 100 per cent of those cadets who were susceptible developed rubella after the introduction of infection into the college.

Second attacks of rubella

13. Second attacks of rubella were found to be rare. Only one of 106 immune pregnant mothers developed a second attack of rubella following exposure. The clinical diagnosis was verified by virus isolation but neither in this woman nor the 105 others did a four-fold rise in antibody titre occur.

Subclinical attacks

14. (a) The number of subclinical attacks of rubella in the communities studied was probably considerable as judged by the proportion (88 per cent) of women who possessed antibody to rubella but who did not give a past history of clinical infection.

(b) Eleven per cent of uninoculated women and 14 per cent of uninoculated men who developed rubella had a subclinical infection.

(c) Forty-eight per cent of the women who developed rubella after immunoglobulin had a subclinical infection. Immunoglobulin, therefore, may suppress the clinical features of the disease but not prevent infection.

Isolation of the rubella virus

15. Rubella virus was isolated in about 50 per cent of specimens obtained from cases of rubella during the first three days after the onset of the rash. During the next three days the virus was isolated from about 25 per cent of cases; thereafter it was seldom isolated.

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4. I attach reprints of two papers reporting certain aspects of this work.

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VOLUME II

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APPENDIX V ix

Examples of protocols, record cards, punch cards, coding sheets, and standard letters.

TABLES AND FIGURES

APPENDIX I

COLLECTION AND HANDLING OF DATA

Once the clinical details and laboratory findings were recorded, the completed cards were returned to the Epidemiological Research Laboratory at the Central Public Health Laboratory, Colindale, London, from the various virus laboratories participating in the studies. Each card was checked on receipt at Colindale so that inaccurate or incomplete information could be detected. Such cards were returned to the appropriate doctor and his help requested.

For all the studies, apart from that conducted at the Metropolitan Police Training College, in which the amount of information was small enough to be conveniently analysed by hand, the record cards were coded. Coding sheets were prepared to enable the data on the cards to be transferred into a numerical code. Once completed the code was checked by a person other than the one who performed the original coding. In this way errors were more likely to be detected than if the coder checked his own cards.

Punch cards were then prepared from the coded information. The punch cards were of the type manufactured by International Computers Ltd. and each contained 80 columns (i.e. the card was able to record up to 80 items of information). As already described for the coding of the record cards, an independent person checked each punch card using a verifier.

These various procedures allowed the relevant information to be extracted by means of a counter-sorter.

APPENDIX II

COLLECTION AND TRANSPORT OF SPECIMENS

a) Blood

In the investigations described, venous blood samples of between 2 and 5 ml. were obtained. Where paired samples were required the first specimen was taken as soon after contact with the case of rubella as possible and the second five to six weeks later. This interval allowed for the incubation period and development of antibody in a susceptible person who became infected. The specimens were sent to the appropriate virus laboratory by the quickest available means; in most instances this was by letter post. In some cases transport by car was arranged. The specimens were kept in sterile $\frac{1}{2}$ fluid oz. McCartney bottles which were wrapped in wadding and put in stout cardboard containers. The containers were placed in padded envelopes which were then sealed. Thus the possibility of leakage of blood was kept to a minimum (see appendix page xvi).

b) Throat and nasal swabs

The swabs were made of sterile cotton wool and kept in glass test tubes until required.

The majority of patients had their noses and throats swabbed at the time the rash was present. Successful isolation of the rubella virus is more likely at this stage of the disease (Green, Balsamo, Giles, Krugman and Mirick 1965).

Once taken, the swabs were dipped into bijou bottles containing virus transport media, the ends broken off and the caps of the bottles replaced securely.

The virus transport media was prepared under aseptic conditions at the Central Public Health Laboratory, Colindale, London, using the following formula:-

Hank's balanced salt solution	990.0 ml.
20% bovine albumin solution	10.0 ml.
4.4% sodium bicarbonate solution	10.0 ml.

Each bijou bottle contained 4 ml. of the above solution at a PH of 7.0 to 7.2. Phenol red (0.002%) was used as an indicator so that a change in PH could be noticed and the bottle discarded.

The bijou bottles were placed in containers similar to those used for the blood samples. The specimens normally reached the laboratory either by post or by hand within 24 hours of being taken. If there was the possibility of delay the doctor was asked to place the bijou bottles in a refrigerator at $+4^{\circ}C$ until they could be sent to the laboratory.

APPENDIX III

LABORATORY METHODS

In the laboratories taking part in the study, uniform though not necessarily completely identical methods were used. A continuous cell line of rabbit kidney (R.K.13) cells (Beale, Christofinis and Furminger, 1963) was employed and the sensitivity of these cells was checked by repeated titration of known strains of rubella virus. A group of control sera was also circulated among the laboratories for titration so that a uniform standard could be ensured (table 26).

a) Neutralisation test

Serial two-fold or four-fold dilutions of serum in 0.3 ml. volumes were mixed with equal volumes of the 'West Point' strain of rubella virus adapted to grow in R.K. 13 cells and diluted to contain about 100 T.C.D. in 0.1 ml. After incubation at 36°C for 75 minutes the virus-serum mixture in 0.2 ml. volumes was inoculated into each of two R.K. 13 tubes. A positive human serum of known titre was included in each test. Tubes were read on the fourth and sixth or seventh days after inoculation. The degree of cytopathic effect was estimated by direct microscopy. The end-point in the antibody titration was taken as the highest dilution of serum causing complete or almost complete inhibition of the cytopathic effect. Titres were expressed as the initial serum dilution before mixing with virus.

b) Haemagglutination-inhibition test

Sera were absorbed with 25% kaolin for one hour at room temperature and after centrifugation with 50% chick red blood cells for at least

two hours at +4C they were then inactivated at 56°C for 30 minutes (Stewart, Parkman, Hopps, Douglas, Hamilton and Meyer, 1967). Serial four-fold serum dilutions in 0.025 ml. volumes were placed in microhaemagglutination plates and mixed with equal volumes of antigen containing 4 to 8 haemagglutinating units. After holding at room temperature for one hour 0.025 ml. of a 0.16% suspension of chick red blood cells was added to each cup. The plates were refrigerated for three hours and the titres read. Antibody titres were expressed as the highest dilution of serum producing complete inhibition of haemagglutination. Appropriate controls were included in each batch of tests.

II Virus isolation

Virus isolation was based on the method of Hutchinson and Thompson (1965). 0.4 ml. of throat swab extract were inoculated on to a tube of R.K.13 cells containing sodium bicarbonate, rabbit serum, antibiotics and Parkers "199" solution. The tubes were then rolled at 35°C overnight and the medium changed to a maintenance medium of Eagle's solution (Burroughs Wellcome). The tubes were rolled at 35°C, examined and the medium changed twice weekly, those showing the cytopathic effect (C.P.E.) of rubella virus being subcultured. Those showing no cytopathic effect after 14 days were subcultured and the subculture observed for a further fortnight. If after this passage no evidence of rubella

cytopathy was found the specimen was considered negative. Isolates were identified by neutralisation with a rabit antiserum prepared with the "West Point" strain.

APPENDIX IV

IMMUNOGLOBULIN

In the United Kingdom immunoglobulin was made available in 1954 for pregnant women in contact with rubella during early pregnancy. It is distributed in England and Wales by the Public Health Laboratory Service, and in Scotland by the National Blood Transfusion Service. The British product is manufactured by the Blood Products Laboratory, Lister Institute of Preventive Medicine, Elstree, Hertfordshire, and by the Blood Products Unit, South East Regional Blood Transfusion Centre, Edinburgh. Occasionally the supply is supplemented by Dutch, French and Swiss material. The batches of immunoglobulin are usually prepared from citrated plasma by fractionation with ether using the method of Kekwick and Mackay (1954) or by fractionation with ethanol, the method of Kistler and Nitschmann (1962). The solution contains 15g. per cent protein and 1:10,000 thiomersal, a 750 mg. dose being contained in 5.1 ml.

APPENDIX V

EXAMPLES OF PROTOCOLS, RECORD CARDS, PUNCH CARDS CODING SHEETS AND STANDARD LETTERS

Serological survey of adult females

not recently in contact with rubella.

P.H.L.S. FIELD TRIAL.

AN INVESTIGATION INTO THE NATURAL IMMUNITY TO RUBELLA IN VARIOUS SECTIONS OF THE COMMUNITY.

P R O T O C O L

Estimation of the rubella antibody titre has now become a standard procedure in certain laboratories and as it is possible that a rubella vaccine may become available in the next few years, it is important to obtain information on the natural immunity of the population to rubella to enable these vaccines to be used in the most economical and effective way. A Public Health Laboratory Service Working Party has therefore been formed to obtain this information.

OBJECT

To assess the titres of rubella antibody particularly in females of various ages.

GENERAL PLAN

Members of the P.H.L.S. Working Party will arrange to obtain the serum samples in their own area. A standard record card should be completed for each patient. The antibody titre will be estimated at the appropriate Public Health Laboratories and the results analysed at the Epidemiological Research Laboratory at Colindale.

METHOD

1. Members of the Working Party will arrange with local centres (ante-natal clinics would probably be the most likely) to obtain about 5 ml of blood from 100 female patients in each five year age group between 20 and 40 years of age together with 100 samples from patients of various ages under 20 years and the same number from patients over 40 years of age.

2. The blood samples should be sent unfrozen to the laboratory but should titration of the sera be delayed, they should be stored at $4^{\circ}C$, or if there is to be a considerable lapse of time, below $-20^{\circ}C$.

3. Serum dilutions of 4, 16 and 64 will be used in titrations. Titres should refer to the initial serum dilutions.

4. A standard record card (Form 13) will be completed for each patient and returned to the Epidemiological Research Laboratory for analysis.

DR/GVS.
20.9.66.

Protocol.

Rubella Antibody Card

RUBELLA ANTIBODY STUDY

FORM 13

For Office Use Only

1 2 3 4 5

Hospital .....
(if applicable)

6 7 8

Patient's Name .....

Address .....

Spare

9 10

.....

11 12 13

Date of Birth .....

Past History of Rubella (ring) YES / NO / NOT KNOWN

14

If YES, approximate date.....

15 16 17

Number of pregnancies.

18

Number of miscarriages or stillbirths.

19

Signature .....
Doctor / Nurse

Date .....

FOR LABORATORY USE

20 21

Name of Laboratory.

22 23 24

Date specimen taken .....

25

Antibody level

Signature .....
Laboratory Director

Date .....

25. Antibody level -

<4	=	1
4	=	2
8	=	3
16	=	4
32	=	5
64	=	6
>64	=	7

RUBELLA ANTIBODY SURVEY (Card No. 13)

CODING

Box No.

1 - 2	Number allocated to investigation.
3 - 5	Number allocated to the patient.
6 - 8	Spare
9	Nationality - British (including Eire) 1. Indian/Pakistani 2. West Indian 3. European 4. African 5. Other 6. Unknown 9.
10	Spare
11 - 12	Age (i.e. Number of years old at date of blood sample) If age is less than 1 year, put 10 in Box 11 and number of months in Box 12. If age is greater than 10 years, code number of years in boxes 11 and 12. If less than 10 years but greater than 1 year, put 0 in Box 11 and number of years in Box 12.
13	Spare
14	Past History of Rubella - YES = 1. NO = 2. NOT KNOWN= 9.
15 - 16	Number of years before specimen taken. If less than 1 year, put 10 in Box 15 and number of months in Box 16. If greater than 10 years, code number of years in Boxes 15 and 16. If less than 10 years but greater than 1 year, put 0 in Box 15 and number of years in Box 16. If "NO" or Not known, code Boxes 15 and 16 "0" "0".
17	Spare
18	Actual number of pregnancies.
19	Actual number of miscarriages or stillbirths.
20 - 21	Spare.
22	Month of obtaining blood - Jan. = 1, Feb. = 2. etc. Nov. = 11 Dec. = 3.
23	Last digit of year number, i.e. 1966 = 6.
24	Spare

Coding Sheet

Investigation into the prophylactic effect of 750 mg. immunoglobulin when given to women in contact with rubella during the first four months of pregnancy.

Protocol

AN INVESTIGATION INTO THE EFFICACY OF GAMMA GLOBULIN IN THE PREVENTION OF RUBELLA IN PREGNANCY.

P R O T O C O L

INTRODUCTION

Until a vaccine against rubella becomes available gamma globulin will probably remain the usual method of protection of women in contact with the disease during the early months of pregnancy. Doubt still exists, however, about the efficacy of gamma globulin in the prevention of infection and the optimum dose has also still to be determined.

A further problem is that many women already have neutralising antibody to rubella virus in their serum and it may not be necessary to give them gamma globulin for protection. It is only recently that the measurement of the serum rubella antibody titre has become a standard laboratory precedure and it is now being carried out in several laboratories. It should, therefore, be possible to see whether the woman is already protected and if not, to determine if the dose of gamma globulin given is sufficient to prevent infection.

To study these problems a Working Party of the Public Health Laboratory Service has been established and it is hoped to gain the co-operation of those Laboratory Directors responsible for issuing gamma globulin, together with those General Practitioners who are administering it to female rubella contacts in the first three months of pregnancy.

OBJECTS

1. To assess the amount of neutralising antibody to rubella virus in women requesting gamma globulin following contact with rubella during the first three months of pregnancy.

2. To assess the efficacy of gamma globulin in suppressing the development of rubella.

3. To follow up babies born of unprotected mothers to look for evidence of congenital rubella.

GENERAL PLAN

Laboratory Directors receiving a request for gamma globulin to be issued to a patient in the first three months of pregnancy who has been in contact with rubella will arrange with the General Practitioner for a blood sample to be taken for rubella antibody estimation before the gamma globulin is given

The dose of gamma globulin will be taken from a reserved batch of known rubella antibody titre.

Five to six weeks later the General Practitioner will be asked to see the patient again and a second blood sample takem.

An attempt should also be made to confirm the diagnosis in the original case by taking throat and nasal swabs or if not feasible at the time, a blood sample retrospectively which, if negative, will show that infection probably never took place.

If possible, throat and nasal swabs should be taken from babies born of mothers who developed rubella despite gamma globulin, as soon after birth as possible.

SUPPLIES

1. CARDS.

Pregnant Contact Record Card (1) No. 25 (Blue)

To record clinical and laboratory information regarding the pregnant rubella contact.

Pregnant Contact Record Card (2) No. 26 (Pink)

To record the protective effect of gamma globulin and the laboratory results obtained from the second blood sample.

Index Case Record Card No. 27 (Gold)

To record clinical and laboratory information regarding the index case of rubella.

Infant Record Card No. 29 (White)

To record the outcome of the pregnancy and the result of throat and nasal swabs taken from the infant.

2. CONTAINERS. (Usually McConkey ½ fl. oz.)

For collection of the blood samples.

3. VIRUS SWABS AND BIJOU BOTTLES.

For swabbing the throat and nose and transporting the swabs to the laboratory.

4. GAMMA GLOBULIN.

This will be taken from a reserved batch of known rubella antibody titre which will be issued to the Laboratory Directors from the Epidemiological Research Laboratory, Colindale.

METHOD

A. Directors of Public Health Laboratories should arrange with General Practitioners requesting gamma globulin for their patients who have been in contact with rubella during the first three months of pregnancy to:-

1. Take about 5 cc of blood from the patient and send this to the appropriate laboratory, together with form No. 25 (Pregnant Contact Record Card (1).

/3.

2. Give 750 mgm of gamma globulin intramuscularly. This gamma globulin will be taken from the reserved batch.

3. Attempt to confirm the diagnosis by taking throat and nasal swabs from the index case, or alternatively, a sample of 5 ml of blood and return this with form No. 27 (Index Case Record Card) to the laboratory.

4. Arrange to see the contact in five to six weeks' time for a second blood sample of 5 ml. Form No. 26 (Pregnant Contact Record Card (2)) will be sent from the laboratory beforehand requesting this.

5. Record the outcome of the pregnancy and arrange, if possible, to take throat and nasal swabs from the infant at birth or as soon after birth as possible, should the mother not have had antibody when gamma globulin was given. Form No. 29 (Infant Record Card) will be sent to the doctor near the estimated date of birth.

B. Numbering of Cards.

Form No. 25 (Pregnant Contact Record Card (1)) will be stamped with an identifying number. This number should be copied on to each subsequent card issued in respect of the contact, index case or infant. i.e. Forms No. 26, 27 and 29.

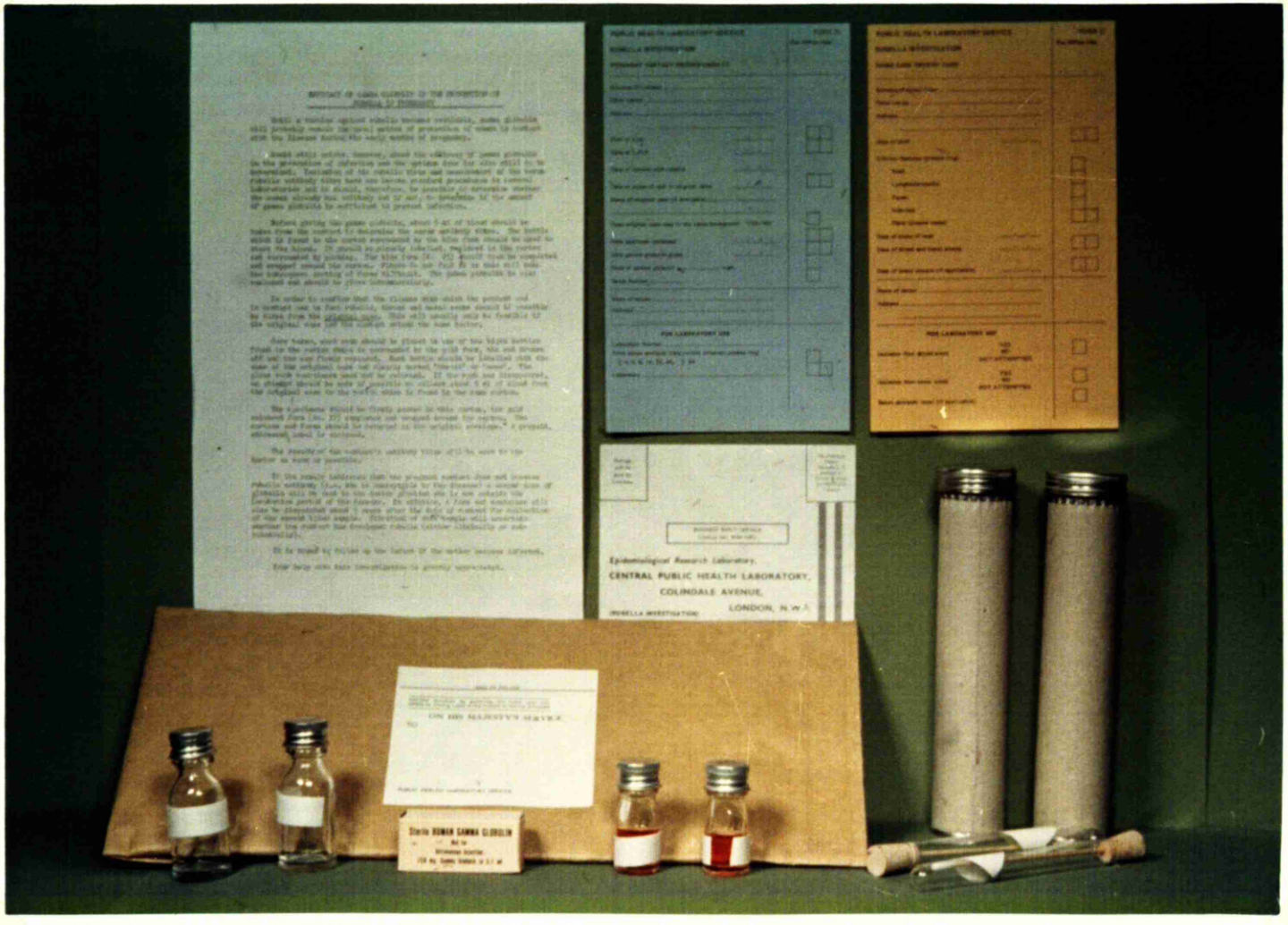
C. The results will be analysed at the Epidemiological Research Laboratory, Central Public Health Laboratory, Colindale Avenue, London, N.W.9.

DR/GVS.
7th November, 1966.

Photograph of the pack sent to general practitioners I.

XVI

Photograph of the pack sent to general practitioners II



EFFICACY OF GAMMA GLOBULIN IN THE PREVENTION OF RUBELLA IN PREGNANCY

Until a vaccine against rubella becomes available, gamma globulin will probably remain the usual method of protection of women in contact with the disease during the early months of pregnancy.

Doubt still exists, however, about the efficacy of gamma globulin in the prevention of infection and the optimum dose has also still to be determined. Isolation of the rubella virus and measurement of the serum rubella antibody titre have now become standard procedures in several laboratories and it should, therefore, be possible to determine whether the woman already has antibody and if not, to determine if the amount of gamma globulin is sufficient to prevent infection.

Before giving the gamma globulin, about 5 ml of blood should be taken from the contact to determine the serum antibody titre. The bottle which is found in the carton surrounded by the blue form should be used to store the blood. It should be clearly labelled, replaced in the carton and surrounded by packing. The blue form (No. 25) should then be completed and wrapped around the carton. Please do not fold it as this will make the subsequent sorting of forms difficult. The gamma globulin is also enclosed and should be given intramuscularly.

In order to confirm that the illness with which the patient was in contact was in fact rubella, throat and nasal swabs should if possible be taken from the original case. This will usually only be feasible if the original case and the contact attend the same doctor.

Once taken, each swab should be placed in one of the bijou bottles found in the carton which is surrounded by the gold form, the end broken off and the cap firmly replaced. Each bottle should be labelled with the name of the original case and clearly marked "throat" or "nose". The glass swab containers need not be returned. If the rash has disappeared, an attempt should be made if possible to collect about 5 ml of blood from the original case in the bottle which is found in the same carton.

The specimens should be firmly packed in this carton, the gold coloured form (No. 27) completed and wrapped around the carton. The cartons and forms should be returned in the original envelope. A prepaid, addressed label is enclosed.

The result of the contact's antibody titre will be sent to the doctor as soon as possible.

If the result indicates that the pregnant contact does not possess rubella antibody (i.e. she is susceptible to the disease) a second dose of globulin will be sent to the doctor provided she is not outside the incubation period of the disease. In addition, a form and container will also be dispatched about 5 weeks after the date of contact for collection of the second blood sample. Titration of this sample will ascertain whether the contact has developed rubella (either clinically or sub-clinically).

It is hoped to follow up the infant if the mother becomes infected.

Your help with this investigation is greatly appreciated.

Instructions sent to the general practitioner with the pack

Pregnant Contact Record Card (1)

a) Front

PUBLIC HEALTH LABORATORY SERVICE — **FORM 25**

For Office Use

RUBELLA INVESTIGATION

PREGNANT CONTACT RECORD CARD (1)

Surname of Contact .....

Other names .....

Address .....

.....

Date of birth ...../...../.....

Date of L.M.P. ...../...../.....

Date of contact with rubella ...../...../.....

Date of onset of rash in original case ...../...../.....

Name of original case (if available).....

.....

Does original case stay in the same household? YES/NO

Date specimen collected ...../...../.....

Date gamma globulin given ...../...../.....

Dose of gamma globulin ..... mgm.

Batch Number .....

Name of doctor .....

Address .....

.....

FOR LABORATORY USE

Laboratory Number .....

First serum antibody titre (initial dilution) please ring

< 4, 4, 8, 16, 32, 64, > 64

Laboratory .....

b) **Reverse**

RUBELLA INVESTIGATION

PREGNANT CONTACT RECORD CARD (I)

Instructions

1. Please give details on this form of each patient who is in contact with rubella during the first ~~three~~ four months of pregnancy.

2. A blood sample of about 5 ml. should be sent with the form.

3. An attempt should be made to confirm the diagnosis of rubella in the original case by taking throat and nasal swabs, or alternatively 5 ml. of blood if the patient has recovered from the disease. All specimens from the original case should be accompanied by form 27.

4. Forms and specimens should be sent to:

Pregnant Contact Record Card (2)

a) **Front**

PUBLIC HEALTH LABORATORY SERVICE — **FORM 26**

RUBELLA INVESTIGATION — **For Office Use**

PREGNANT CONTACT RECORD CARD (2)

Contact's No. .....

Surname of Contact .....

Other names .....

Address .....

.....

Did contact develop rubella? YES / NO ☐

If YES, please give date of onset of rash ...../...../..... ☐☐

Is pregnancy progressing? YES / NO ☐

If NO, please give reason ..... ☐

..... ☐

Date second specimen collected ...../...../..... ☐☐

Name of doctor .....

Address .....

.....

FOR LABORATORY USE

Laboratory number .....

Second serum antibody titre (initial dilution) please ring ☐

< 4, 4, 8, 16, 32, 64, > 64.

b) **Reverse**

RUBELLA INVESTIGATION

PREGNANT CONTACT RECORD CARD (2)

Instructions

1. This form should accompany the second blood sample of 5 ml. taken from a patient who had been given gamma globulin for protection against rubella 5 to 6 weeks previously.

2. The form and specimen should be sent to:

Index Case Record Card

a) **Front**

PUBLIC HEALTH LABORATORY SERVICE — **FORM 27**

RUBELLA INVESTIGATION — **For Office Use**

INDEX CASE RECORD CARD

Surname of Index Case .....

Other names .....

Address .....

.....

Date of birth ...../...../.....

Clinical features (please ring).

- Rash
- Lymphadenopathy
- Fever
- Arthritis
- Other (please state)

Date of onset of rash ...../...../.....

Date of throat and nasal swabs ...../...../.....

Date of blood sample (if applicable) ...../...../.....

Name of doctor .....

Address .....

.....

FOR LABORATORY USE

Isolation from throat swab — YES / NO / NOT ATTEMPTED

Isolation from nasal swab — YES / NO / NOT ATTEMPTED

Serum antibody level (if applicable)

b) **Reverse**

RUBELLA INVESTIGATION

INDEX CASE RECORD CARD

Instructions

1. Please give details on this form of the index case of rubella who was in contact with the pregnant patient.

2. Throat and nasal swabs in separate bijou bottles should be taken as soon as possible, or alternatively a blood sample of 5 ml. if the patient has recovered from the disease.

3. The form and specimen should be sent to:

Pre-paid Label

Postage will be paid by Licensee

No Postage Stamp necessary if posted in Great Britain or Northern Ireland

BUSINESS REPLY SERVICE
Licence No. NW 1473

Epidemiological Research Laboratory,

CENTRAL PUBLIC HEALTH LABORATORY,

COLINDALE AVENUE,

LONDON, N.W.9.

(RUBELLA INVESTIGATION)

Box No.

18 Dose of Gamma Globulin.

< 750 mgm	=	1
750 "	=	2
> 750 - <1500	=	3
1500	=	4
>1500 - <2250	=	5
2250	=	6
>2250 - <3000	=	7
3000 and over	=	8
NOT KNOWN	=	9
NOT STATED	=	0

19 Batch Number of Gamma Globulin.

LKG. 121	=	1
123	=	2
127	=	3
131	=	4
138	=	5
141	=	6
143	=	7
Other reserved batches	=	8
Other batches	=	9
NOT KNOWN or NOT STATED	=	D

20 First serum antibody titre.

< 4	=	1
4	=	2
8	=	3
16	=	4
32	=	5
64	=	6
>64	=	7
Not tested	=	8
Not stated	=	9
H.I. test	=	0

21 - ~~22xxxxSpare~~

H.I. test.

<8	=	1
8	=	2
16	=	3
32	=	4
64	=	5
128	=	6
>128	=	7

22 - Spare.

RUBELLA INVESTIGATION - GAMMA GLOBULIN. (Card No. 25 - Blue)

CODING

Box No.

1 - 2 Number allocated to member of Working Party.

3 - 6 Number allocated to rubella contact.

7 - 8 Age (i.e. Number of years old at date of blood sample).

If NOT KNOWN - code 9. 9.

If NOT STATED- code 8. 8.

9 - 10 Number of weeks pregnant at date of contact with rubella.

If greater than 10 weeks, code number of weeks in boxes 9 and 10.

If less than 10 weeks, put 0 in box 9 and number of weeks in box 10.

If NOT KNOWN - code 9. 9.

If NOT STATED- code 8. 8.

If before pregnancy code 7. 7.

11 - 12 Original case of rubella.

Number of days between onset of rash and date of contact.

If greater than 10 days, code number of days in boxes 11 and 12.

If less than 10 days, code 0 in box 11 and number of days in box 12.

If NOT KNOWN - code 9. 9.

If NOT STATED- code 8. 8.

If contact if before date of rash Code 7 in Box 11 and number of days between contact and date of rash in box 12. If this interval is 9 days or greater, code 9 in box 12.

13 Original case of rubella.

Household contact - YES = 1

NO = 2

NOT KNOWN = 9

NOT STATED = 8

14 - 15 Number of days specimen collected after contact with rubella.

If greater than 10 days, code number of days in boxes 14 and 15.

If less than 10 days, put 0 in box 14 and number of days in box 15.

If NOT KNOWN - code 9. 9.

If NOT STATED- code 8. 8.

16 - 17 Date gamma globulin given.

Number of days after contact with rubella.

If greater than 10 days, code number of days in boxes 16 and 17.

If less than 10 days, code 0 in box 16 and number of days in box 17.

If NOT KNOWN,- code 9. 9.

If NOT STATED- code 8. 8.

RUBELLA INVESTIGATION - GAMMA GLOBULIN. (Card No. 26 - Pink)

CODING

Box No.

23 Development of rubella in the contact.

YES	=	1
NO	=	2
NOT KNOWN	=	9
NOT STATED	=	8

24 - 25 If rash developed in contact, code number of days after administration of gamma globulin.

If less than 10 days, put 0 in box 24 and number of days in box 25.

If 10 days or greater, code number of days in boxes 24 and 25.

If NOT KNOWN - code 9. 9.
If NOT STATED - code 8. 8.

26 Continuation of pregnancy.

YES	=	1
NO	=	2
NOT KNOWN	=	9
NOT STATED	=	8

27 Reasons for pregnancy NOT progressing.

NEVER PREGNANT	=	1
MISCARRIAGE	=	2
THERAPEUTIC ABORTION	=	3
OTHER REASON	=	4
NOT KNOWN	=	9
NOT STATED	=	8

28 Spare

29-30 Number of weeks after first specimen.

If 10 weeks or greater, code number of weeks in boxes 29 and 30.

If less than 10 weeks, put 0 in box 29 and number of weeks in box 30.

31 Rise in titre.

NO CHANGE	=	0
1 Tube difference	=	1
2 Tubes "	=	2
3 " "	=	3
4 " "	=	4

Cont'd...

Box No.

31 Cont'd.. Rise in titre.

5 tubes difference	=	5
6 " "	=	6
7 " "	=	7
NO BLOOD SUBMITTED	=	8
OTHER REASON	=	9

DR/GVS. 6.7.67.

RUBELLA INVESTIGATION - GAMMA GLOBULIN. (Card No. 27-Gold)

CODING

Box No.

32 - 33 Age (i.e. Number of years old at date of illness).

If age is less than 1 year, code 10 in box 32 and number of months in box 33.

If age is 10 years or greater, code number of years in boxes 32 and 33.

If age is less than 10 years but greater than 1 year, code 0 in box 32 and number of years in box 33.

34 Rash.

If present = 1

If absent = 2

35 Lymphadenopathy

If present = 1

If absent = 2

36 Fever.

If present = 1

If absent = 2

37 Arthritis

If present = 1

If absent = 2

38 Other

If present = 1

If absent = 2

39 Spare

40 - 41 Throat and nasal swabs.

If swabs taken but date unknown, code 8.8. in boxes 40. 41.

Number of days after rash (or other feature of illness).

If 10 days or greater, code number of days in boxes 40 and 41.

If not taken code 9. 9.

If less than 10 days, code 0 in box 40 and number of days in box 41.

42 - 43 Blood sample.

If taken but date unknown code 8. 8. in boxes 42. 43.

Number of days after the rash (or other feature of illness).

If not taken, code 9. 9.

If greater than 10 days, code number of days in boxes 42 and 43.

If less than 10 days, code 0 in box 42 and number of days in box 43.

Box No.

44 Throat swab isolation.

YES	=	1
NO	=	2
NOT ATTEMPTED	=	3
NOT STATED	=	8

45 Nasal swab isolation.

YES	=	1
NO	=	2
NOT ATTEMPTED	=	3
NOT STATED	=	8

46 Serum antibody level.

< 4	=	1
4	=	2
8	=	3
16	=	4
32	=	5
64	=	6
> 64	=	7
NOT STATED	=	8
BLOOD NOT SUBMITTED	=	9

DR/GVS. 6.7.67.

Study of the attack rate of rubella in female contacts of child-bearing age who were not given immunoglobulin.

AN INVESTIGATION INTO VARIOUS EPIDEMIOLOGICAL ASPECTS OF RUBELLA

P R O T O C O L

INTRODUCTION

Although gamma globulin has been employed extensively in this and other countries for the protection of women exposed to rubella during the first trimester of pregnancy, its value has never been assessed adequately.

During the past year the Public Health Laboratory Service has been collaborating with many general practitioners in investigating the protective effect of gamma globulin. The general plan has been to study the incidence of rubella in susceptible pregnant women in contact at home who have been treated with gamma globulin. It is essential at this stage to obtain precise information about the incidence of rubella in a control group of women exposed in the home who are not given gamma globulin.

GENERAL PLAN

The investigation will include any woman of child-bearing age who is not pregnant and is in contact in the home with a patient with rubella. To establish the diagnosis a throat and nasal swab should be obtained from the patient and sent to the appropriate Public Health Laboratory accompanied by a record card containing clinical details. At the same time each woman of child-bearing age in the home should have a serum sample withdrawn to determine susceptibility. This serum sample should also be sent to the laboratory with the appropriate record card.

If the sample is shown to lack antibody a second specimen will be requested six weeks later to determine whether the contact has become infected.

SUPPLIES

The following items will be issued by the Public Health Laboratory Service:-

1. RECORD CARDS

   Index Case Record Card No. 56.

   To record details of a case of rubella.

   Contact Record Card (1) No. 57.

   To record details of a female household contact of between 15 and 45 years of age.

   Contact Record Card (2) No. 58.

   This card will be sent only if the contact is shown to be susceptible (i.e. lacks antibody in the first specimen).

2. CONTAINERS FOR BLOOD (usually ½ fl. oz. McCartney bottles).

   For collection of blood samples.

3. VIRUS SWABS AND BIJOU BOTTLES CONTAINING TRANSPORT MEDIUM.

For swabbing the throat and nose and for transport of the swabs.

SPECIMENS

1. Throat and Nasal Swabs.

These should be taken from the nose and throat as soon as possible from any case of rubella which occurs in a household. The swabs should then be dipped into separate bijou bottles containing virus transport medium, the ends broken off and the caps replaced. The label should be marked throat or nose.

2. Blood.

Up to 5 ml of blood should be taken, placed in a McCartney bottle and sent unrefrigerated.

METHOD

1. A record will be made by the doctor on the Index Case Record Card of any case of rubella which occurs in a household in his practice.

2. Throat and nasal swabs should be taken from the case as soon as possible.

The specimens should be sent by letter post together with the record card (No. 56) to the appropriate laboratory.

Should there be any delay, the swabs should be kept refrigerated at around +4°C until posted.

3. All female home contacts of between 15 and 45 years of age should be bled, the specimen to be taken as soon after contact as possible. This specimen should be sent to the laboratory accompanied by Contact Record Card (1).

4. This sample will be titrated for rubella antibody at the laboratory and if found to be negative the doctor will be sent a request for the second blood sample six weeks later, accompanied by Contact Record Card (2).

5. Numbering of Cards.

A number will be stamped on to the Contact Record Card (1). To facilitate analysis and identification of the case and contact, it would be appreciated if this number is copied on to all other cards pertaining to the contact.

DR/GVS.
20.11.67.

Protocol

Contact Record Card (1)

a) **Front**

PUBLIC HEALTH LABORATORY SERVICE

RUBELLA INVESTIGATION

CONTACT RECORD CARD (1)

FORM 57
For Office Use

Surname of Contact .....

Other names .....

Address .....

.....

Date of birth ...../...../.....

Date of contact with rubella ...../...../.....

Date of onset of rash in original case ...../...../.....

Name of original case (if available).....

.....

Does original case stay in the same household? YES/NO

Date specimen collected ...../...../.....

Name of doctor .....

Address .....

.....

FOR LABORATORY USE

Laboratory Number .....

First serum antibody titre (initial dilution) please ring

< 4, 4, 8, 16, 32, 64, > 64

Laboratory .....

b) **Reverse**

RUBELLA INVESTIGATION

CONTACT RECORD CARD (I)

Instructions

1. Please give details on this form of each female patient (between the ages of 15 and 45 years) who is in contact with rubella.

2. A blood sample of about 5 ml should be sent with the form.

3. An attempt should be made to confirm the diagnosis of rubella in the original case by taking throat and nasal swabs. All specimens from the original case should be accompanied by Form 56.

4. Forms and specimens should be sent to:

Contact Record Card (2)

a) **Front**

PUBLIC HEALTH LABORATORY SERVICE

RUBELLA INVESTIGATION

CONTACT RECORD CARD (2)

FORM 58

For Office Use

Contact's No. .....

Surname of Contact .....

Other names .....

Address .....

.....

Did contact develop rubella? YES / NO ☐

If YES, please give date of onset of rash. ...../...../..... ☐☐

Date second specimen collected ...../...../..... ☐☐

Name of doctor .....

Address .....

.....

FOR LABORATORY USE

Laboratory number .....

Second serum antibody titre (initial dilution) please ring

< 4, 4, 8, 16, 32, 64, > 64. ☐

b) **Reverse**

RUBELLA INVESTIGATION

CONTACT RECORD CARD (2)

Instructions

1. This form should accompany the second blood sample of 5 ml taken from a patient who had been in contact with rubella.

2. The form and specimen should be sent to:

Index Case Record Card

a) **Front**

PUBLIC HEALTH LABORATORY SERVICE

RUBELLA INVESTIGATION

INDEX CASE RECORD CARD

FORM 56

For Office Use

Surname of Index Case .....

Other names .....

Address .....

.....

Date of birth ...../...../.. .....

Clinical features (please ring).

- Rash
- Lymphadenopathy
- Fever
- Arthritis
- Other (please state)

Date of onset of rash ...../...../.....

Date of throat and nasal swabs ...../...../.....

Name of doctor .....

Address .....

.....

FOR LABORATORY USE

Isolation from throat swab — YES / NO / NOT ATTEMPTED

Isolation from nasal swab — YES / NO / NOT ATTEMPTED

b) **Reverse**

RUBELLA INVESTIGATION

INDEX CASE RECORD CARD

Instructions

1. Please give details on this form of the index case of rubella who may have infected the female contact. .

2. Throat and nasal swabs in separate bijou bottles should be taken as soon as possible, or alternatively a blood sample of 5 ml if the patient has recovered from the disease.

3. To facilitate subsequent analysis the number given on form 57 should be copied on to the top right hand corner of this card.

4. The form and specimens should be sent to:

Coding Sheet

CODING

Box No.

1 - 2 Number allocated to member of Working Party.

3 - 6 Number allocated to rubella contact.

7 - 8 Age (i.e. Number of years old at date of blood sample).

If NOT KNOWN - code 9. 9.

If NOT STATED - code 8. 8.

9 - 10 Original case of rubella

Number of days between onset of rash and date of contact.

If greater than 10 days, code number of days in boxes 9 and 10.

If less than 10 days, code 0 in box 9 and number of days in box 10.

If NOT KNOWN - code 9. 9.

If NOT STATED - code 8. 8.

If contact is before date of rash: code 7 in box 9 and number of days between contact and date of rash in box 10. If this interval is 9 days or greater, code 9 in box 10.

11 Original case of rubella

Household contact - YES = 1

NO = 2

NOT KNOWN = 9

NOT STATED = 8

12 - 13 Number of days specimen collected after contact with rubella.

If greater than 10 days, code number of days in boxes 12 and 13.

If less than 10 days, put 0 in box 12 and number of days in box 13.

If NOT KNOWN - code 9. 9.

If NOT STATED - code 8. 8.

14 First serum antibody titre. (Neutralisation Test)

< 4	=	1
4	=	2
8	=	3
16	=	4
32	=	5
64	=	6
>64	=	7
Not tested	=	8
Not stated	=	9
H.I. Test	=	0

Cont'd.....

15 H.I. Test

<8	=	1
8	=	2
16	=	3
32	=	4
64	=	5
128	=	6
>128	=	7

16 Spare.

RUBELLA INVESTIGATION - CONTROL GROUP (Card No. 58 Blue)

CODING

Box No.

17 Development of rubella in the contact.

YES	=	1
NO	=	2
NOT KNOWN	=	9
NOT STATED	=	8

18 - 19 If rash developed in contact, code number of days after contact with index case.

If less than 10 days, put 0 in box 18 and number of days in box 19.

If 10 days or greater, code number of days in boxes 18 and 19.

If NOT KNOWN - code 9. 9.

If NOT STATED - code 8. 8.

20 - 21 Number of weeks after first specimen

If 10 weeks or greater code number of weeks in boxes 20 and 21.

If less than 10 weeks, put 0 in box 20 and number of weeks in box 21.

22 Rise in titre.

NO CHANGE	=	0
1 Tube difference	=	1
2 Tubes "	=	2
3 " "	=	3
4 " "	=	4
5 " "	=	5
6 " "	=	6
7 " "	=	7
NO BLOOD SUBMITTED	=	8
OTHER REASON	=	9

CODING

Box No.

23 - 24 Age (i.e. Number of years old at date of illness).

If age is less than 1 year code 10 in box 23 and number of months in box 24
If age is 10 years or greater code number of years in boxes 23 and 24

If age is less than 10 years but greater than 1 year, code 0 in box 23 and number of years in box 24.

25 Rash

If present = 1

If absent = 2

If NOT STATED = 8

26 Lymphadenopathy

If present = 1

If absent = 2

If NOT STATED = 8

27 Fever

If present = 1

If absent = 2

If NOT STATED = 8

28 Arthritis

If present = 1

If absent = 2

If NOT STATED = 8

29 Other

If present = 1

If absent = 2

If NOT STATED = 8

30 Spare

31 - 32 Throat and nasal swabs

Number of days after rash (or other feature of illness).

If 10 days or greater, code number of days in boxes 31 and 32.

If less than 10 days, code 0 in box 31 and number of days in box 32.

If swabs taken, but date unknown, code 8. 8. in boxes 31 - 32.
If not taken code 9. 9.

Cont'd....

Box No.

33 - 34 Blood sample.

Number of days after the rash (or other feature of illness).

If greater than 10 days, code number of days in boxes 33 and 34.

If less than 10 days, code 0 in box 33 and number of days in box 34.

If taken but date unknown code 8. 8. in boxes 33 - 34.

If not taken code 9. 9.

35 Throat swab isolation

YES	=	1
NO	=	2
NOT ATTEMPTED	=	3
NOT STATED	=	8

36 Nasal swab isolation.

YES	=	1
NO	=	2
NOT ATTEMPTED	=	3
NOT STATED	=	8

37 Serum antibody level. (Neutralisation Test)

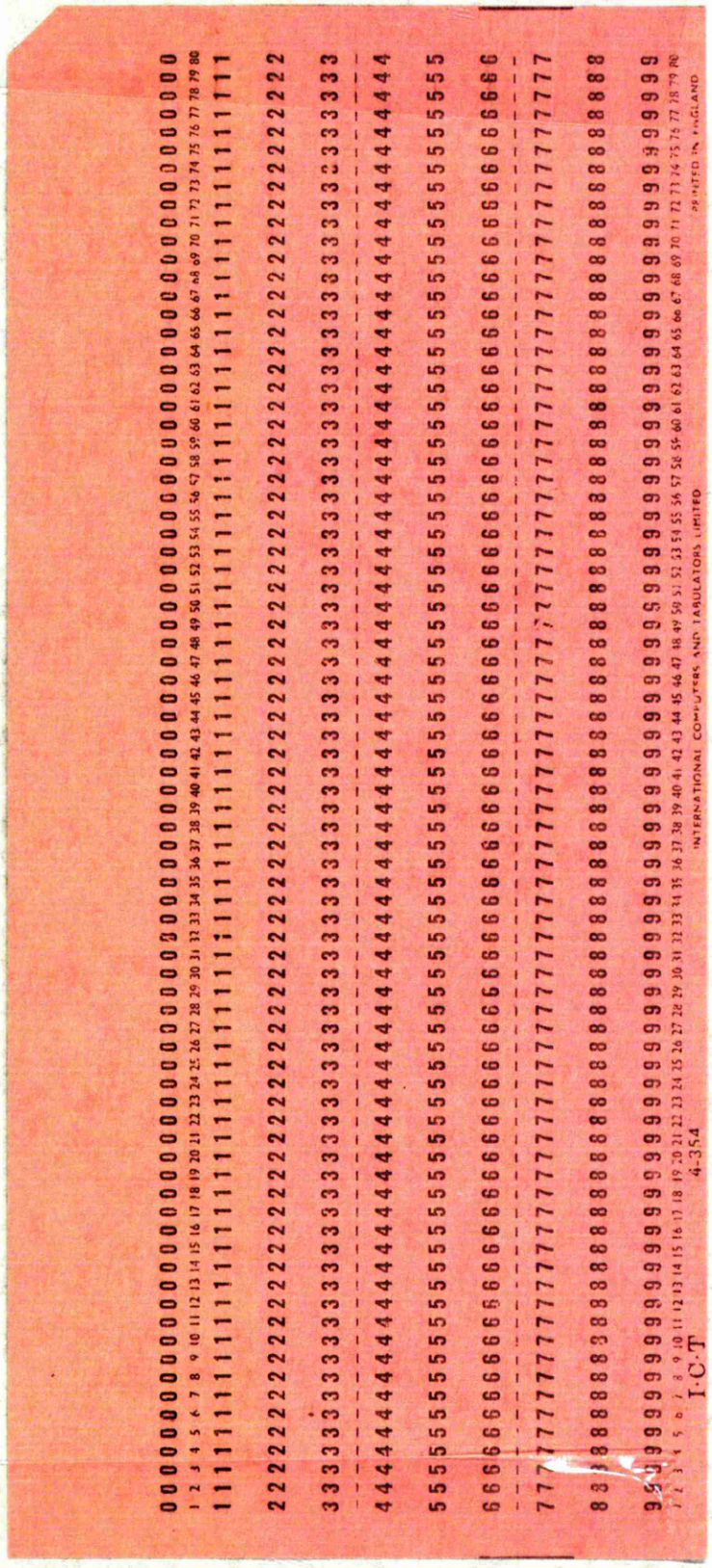
< 4	=	1
4	=	2
8	=	3
16	=	4
32	=	5
64	=	6
>64	=	7
NOT STATED	=	8
BLOOD NOT SUBMITTED	=	9
H. I. Test	=	0

38 H.I. Test

< 8	=	1
8	=	2
16	=	3
32	=	4
64	=	5
128	=	6
>128	=	7

Serological evidence = 8
J Mbelle

Punch Card



(Public Health Laboratory Service)

Telephone: 01 - 205 7041
Telegrams: DEFENDER, LONDON, N.W.9.

COLINDALE AVENUE,
LONDON, N.

Dear Dr.

Assessment of Gamma Globulin in the Prevention of Rubella in Pregnancy.

I enclose a report on your patient's antibody titre to rubella. Th information will be of great help in assessing the value of gamma globuli Thank you for sending it to us.

We are continuing to examine specimens from women given gamma globu and will be very glad if you will continue to send these samples. Meanwh in order to make an adequate assessment of this substance, it is essentia have information on the incidence of rubella in an adequate control group can best be obtained by a parallel study in women of child-bearing age, i contact with a case of rubella in the home, but who are not pregnant and therefore not given gamma globulin.

We should be most grateful if you could help with this phase of the investigation if the opportunity arises, by sending the following to us :

1. Throat and nasal swabs from a suspected case of rubella.
2. A blood sample (2-5 ml) from any non-pregnant woman of child-bearing age who is in contact with the case at home.

   As with the present sample we will send you the antibody titr which you may like to know for any subsequent pregnancy. If woman is shown to possess antibody to rubella (usually over 8 of women have rubella antibody), no further specimens will be required, but if no antibody is found, we should like to send a second specimen to be taken about six weeks after the first see if she has become infected.

I enclose more detailed information together with record cards and containers for the samples. Further packs can be sent as required.

Any help you can give will be greatly appreciated. Please let me k if I can provide any further information.

Yours sincerely,

Encls:

D. Reid.

Letter sent to general practitioners requesting their help to augment the control group

TABLES

Table

1. Defects reported in infants whose mothers had rubella during the first sixteen weeks of pregnancy.
2. Rubella neutralising antibody titres found in women living in different areas of the survey.
3. Rubella neutralising antibody titres at different ages.
4. Rubella neutralising antibody titres according to place of origin.
5. Rubella neutralising antibody titres according to the past history of rubella.
6. Rubella neutralising antibody titres according to the number of pregnancies per patient.
7. Rubella neutralising antibody titres according to the number of miscarriages and stillbirths per patient.
8. Rubella neutralising antibody titres in 15 batches of human immunoglobulin.
9. Attack rate of rubella among pregnant women given 750 mg. immunoglobulin after contact with a case of clinical rubella.
10. Rubella among pregnant women given 750 mg. immunoglobulin after contact with a case of clinical rubella either inside or outside the home.

Table

11. Attack rate of rubella among pregnant women given 750 mg. immunoglobulin after contact with an index case from whom rubella virus was isolated.

12. Rubella among pregnant women who did not possess antibody to rubella and who were given 750 mg. immunoglobulin after contact with an index case from whom rubella virus was isolated.

13. Interval between date of onset of rash in index case and development of rash in 12 home contacts given 750 mg. immunoglobulin - rubella virus isolated from index case.

14. Age distribution of the pregnant rubella contacts according to the possession of antibody to rubella.

15. Attack rate of rubella among susceptible pregnant household contacts given a second dose of immunoglobulin (1,500 mg.) after contact with an index case.

16. Attack rate of rubella among female household contacts of clinical rubella who were not pregnant and who did not receive immunoglobulin.

17. Attack rate of rubella among female household contacts of rubella who were not pregnant and did not receive immunoglobulin - rubella virus isolated from index case.

Table

18. Interval between the date of onset of the rash in index case and the development of rash in 13 home contacts who were not given immunoglobulin - rubella virus isolated from index case.

19. Age distribution of the non-pregnant rubella contacts according to the possession of antibody to rubella.

20. Clinical features of the index cases diagnosed clinically as rubella.

21. Interval (days) between the date of onset of rash in the index case of rubella and the date of taking the swab, according to the isolation of the rubella virus.

22. Rubella haemagglutination-inhibiting antibody titres in 224 Metropolitan Police Cadets according to area of origin.

23. Rubella haemagglutination-inhibiting antibody titres in 224 Metropolitan Police Cadets and the development of rubella.

24. Rubella haemagglutination-inhibiting antibody titres and dates of onset of rash in seven Metropolitan Police Cadets who did not possess antibody when in contact with cases of rubella.

Table

25. Frequency of rising neutralising or haemagglutination-inhibiting antibody titres in women with pre-existing antibody to rubella who were in contact with laboratory-confirmed cases of rubella.

26. Rubella neutralising antibody in the control sera: results of tests by members of the Working Party.

Table 1

Defects reported in infants whose mothers had rubella during the first sixteen weeks of pregnancy

Cardiovascular

- Patent ductus arteriosus
- Septal defects of the heart
- Fallot's tetralogy
- Pulmonary stenosis and coarctation
- Renal artery stenosis
- Myocardial necrosis

Reticulo-endothelial

- Hepatosplenomegaly
- Cholestasis
- Hepatic necrosis
- Giant cell hepatitis
- Splenic fibrosis

Ophthalmic

- Cataracts
- Microphthalmia
- Pigmentary retinopathy
- Glaucoma
- Corneal clouding
- Strabismus
- Nystagmus
- Coloboma
- Chorioretinitis

Nervous

- Encephalitis
- Microcephaly
- Retardation
- "Cerebral palsy"

Auditory

- Maldevelopment of organ of Corti
- Cochlear degeneration
- Stapes fixation

Miscellaneous

- Interstitial pneumonitis
- Osseous rarefaction
- Oesophageal atresia
- Hypospadias
- Growth retardation
- Abnormal dermatoglyphics

Haemopoietic

- Thrombocytopenic purpura
- Anaemia - normocytic
  hypoplastic
  haemolytic
- Reticulocytosis
- Normoblastaemia
- Abnormal red cell morphology
- Lymphadenopathy
- Hypogammaglobulinaemia

Table 2

Rubella neutralising antibody titres found in women living in the different areas of the survey

Area	Rubella neutralising antibody titres*					Totals
	<4	4	8 - 16	32 - 64	>64	
Bedford	12 (8%)	6 (4%)	41	54	36	149
Bristol	18 (18%)	0	38	42	0	98
Carmarthen	9 (14%)	11 (17%)	18	22	5	65
Coventry	8 (5%)	1 (1%)	29	80	29	147
Leeds/Keighley	26 (4%)	6 (1%)	213	380	10	635
Liverpool	5 (17%)	1 (3%)	12	8	3	29
London	38 (20%)	0	77	71	6	192
Manchester	61 (9%)	15 (2%)	297	220	57	650
Newcastle	7 (17%)	4 (9%)	18	13	0	42
Totals	184 (9%)	44 (2%)	743 (37%)	890 (44%)	146 (7%)	2007

* Rubella neutralising antibody titres expressed as the reciprocal of the highest dilution of serum producing complete or almost complete inhibition of the cytopathic effect.

Table 3

Rubella neutralising antibody titres at different ages

Age (Years)	Rubella neutralising antibody titres *			Totals
	<4	4	>8	
15 - 19	58 (16%)	8	302 (82%)	368
20 - 24	43 (8%)	19	500 (88%)	562
25 - 29	44 (9%)	6	417 (90%)	467
30 - 34	20 (7%)	5	276 (92%)	301
35 - 39	18 (9%)	4	171 (89%)	193
40 - 44	1 (1%)	2	113 (97%)	116

* Reciprocal titres of initial serum dilutions

Table 4

Rubella neutralising antibody titres according to place of origin

Place of origin	Rubella neutralising antibody titres *		
	<4	4	⩾8
United Kingdom	153 (9%)	41	1593 (89%)
Europe	2 (7%)	2	26 (85%)
India/Pakistan	2 (4%)	0	43 (95%)
West Indies	16 (21%)	0	61 (78%)
Africa	5 (33%)	1	9 (60%)
Other	6 (11%)	0	47 (89%)

* Reciprocal titres of initial serum dilutions

Table 5

Rubella neutralising antibody titres according to the past history of rubella

Past history of rubella	Rubella neutralising antibody titres *					Totals
	<4	4	8 - 16	32 - 64	>64	
Yes	26 (5%)	6 (1%)	190	278	45	545
No	127 (12%)	30 (3%)	426	445	72	1100
Not known	31 (9%)	8 (2%)	127	167	29	362

* Reciprocal titres of initial serum dilutions

Table 6

Rubella neutralising antibody titres according to the number of pregnancies per patient

Number of pregnancies per patient	Rubella neutralising antibody titres *					Totals
	<4	4	8 - 16	32 - 64	>64	
Nil	5 (29%)	0	3	7	2	17
1	72 (10%)	23 (3%)	275	303	62	735
2	44 (9%)	10 (2%)	176	220	23	473
3	26 (8%)	3 (1%)	114	137	18	298
4	15 (8%)	3 (2%)	71	91	19	199
5	5 (5%)	0	44	50	9	108
⩾ 6	17 (10%)	5 (3%)	60	82	13	177

* Reciprocal titres of initial serum dilutions

Table 7

Rubella neutralising antibody titres according to the number of miscarriages and stillbirths per patient

Number of miscarriages or stillbirths per patient	Rubella neutralising antibody titres *					Totals
	< 4	4	8 - 16	32 - 64	> 64	
Nil	150 (9%)	39 (2%)	607	685	115	1596
1	23 (9%)	4 (1%)	94	132	20	273
2	5 (7%)	0	23	40	4	72
3	1 (5%)	0	5	10	2	18
4	1 (9%)	0	1	6	3	11
Not known	4 (11%)	1 (3%)	13	17	2	37

* Reciprocal titres of initial serum dilutions

Table 8

Rubella neutralising antibody titres in 15 batches of human immunoglobulin *

Batch number	Reciprocal titres of neutralising antibody
LKG 106	240
107	160
108	120
109	120
121	240
123	320
125	80
127	120
129	120
131	320
137	80
138	120
140	60
141	120
143	120

* Manufactured by the Lister Institute of Preventive Medicine

Table 9

Attack rate of rubella among pregnant women given 750 mg immunoglobulin after contact with a case of clinical rubella

Total number of contacts			1747
Number with antibody to rubella*			1483 (85%)
Number without antibody to rubella			264 (15%)
Number who developed[x] rubella	Clinical	24	46
	Subclinical	22	

* Reciprocal titres of > 4 by the neutralisation test or > 8 by the haemagglutination-inhibition test

x Four-fold or greater rise in rubella antibody titre

Table 10

Rubella among pregnant women given 750 mg immunoglobulin after contact with a case of clinical rubella either inside or outside the home

Total number of contacts who developed rubella		46
a) With household index case		28 (61%)
Clinical illness	14	
Subclinical illness	14	
b) With outside index case		18 (39%)
Clinical illness	10	
Subclinical illness	8	

Table 11

Attack rate of rubella among pregnant women given 750 mg immunoglobulin after contact with an index case from whom rubella virus was isolated

Number of pregnant contacts	126	
Number with antibody to rubella	101	(80%)
Number without antibody to rubella	25	(20%)
Number who developed rubella	14	
Clinical	11	
Subclinical	3	

Table 12

Rubella among pregnant women who did not possess antibody to rubella and who were given 750 mg immunoglobulin after contact with an index case from whom rubella virus was isolated

Index case in same household (75 instances)		
Number of pregnant contacts without antibody		15
Number who developed rubella		12
	Clinical 9	
	Subclinical 3	
Index case not in same household (51 instances)		
Number of pregnant contacts without antibody		10
Number who developed rubella		2
	Clinical 2	
	Subclinical 0	

Table 13

Interval between date of onset of rash in index case and development of rash in 12 home contacts given immunoglobulin - rubella virus isolated from index case

Interval (days) between the onset of rashes in the index case and the home contact given immunoglobulin	Number of patients
10 - 12	1
13 - 15	2
16 - 18	5
19 - 21	0
22 - 24	1
25 +	0
Subclinical	3

Table 14

Age distribution of the pregnant rubella contacts according to the possession of antibody to rubella

Age (Years)	Number of contacts with antibody	Number of contacts without antibody	Totals
15 - 19	57 (89%)	7	64
20 - 24	418 (80%)	102	520
25 - 29	539 (84%)	98	637
30 - 34	341 (88%)	46	387
35 - 39	86 (91%)	9	95
40 - 44	18 (94%)	1	19
Not known	24 (96%)	1	25

Table 15

Attack rate of rubella among susceptible pregnant household contacts given a second dose of immunoglobulin (1500 mg) after contact with an index case

Isolation of rubella virus from index case	Number of contacts who developed rubella		Number of contacts who did not develop rubella
	Clinically	Subclinically	
Yes	4 (66%)	0	2
No	3 (6%)	7 (14%)	41
Total	7 (12%)	7 (12%)	43

Table 16

Attack rate of rubella among female household contacts of clinical rubella who were not pregnant and did not receive immunoglobulin

Number of contacts	543	
Number with antibody to rubella	493	(91%)
Number without antibody to rubella	50	(9%)
Number who developed rubella	18	
Clinical	16	
Subclinical	2	

Table 17

Attack rate of rubella among female household contacts of rubella who were not pregnant and did not receive immunoglobulin - rubella virus isolated from index case

Number of contacts		208
Number with antibody to rubella		182 (87%)
Number without antibody to rubella		26 (13%)
Number who developed rubella		13
	Clinical 11	
	Subclinical 2	

Table 18

Interval between the date of onset of rash in index case and the development of rash in 13 home contacts who were not given immunoglobulin - rubella virus isolated from index case

Interval (days) between onset of rashes in the index case and the uninoculated home contact	Number of patients
10 - 12	2
13 - 15	2
16 - 18	3
19 - 21	3
22 - 24	0
25 +	1
Subclinical	2

Table 19

Age distribution of the non-pregnant rubella contacts according to the possession of antibody to rubella

Age (Years)	Number of contacts with antibody	Number of contacts without antibody	Totals
15 - 19	32 (91%)	3	35
20 - 24	68 (89%)	8	76
25 - 29	120 (88%)	16	136
30 - 34	144 (90%)	16	160
35 - 39	78 (94%)	5	83
40 - 44	36 (97%)	1	37
Not known	15 (94%)	1	16

Table 20

Clinical features of the index cases diagnosed clinically as rubella

Clinical Feature	Number of patients with the feature	Number of patients without the feature
Rash	848 (97%)	26
Lymphadenopathy	716 (82%)	158
Fever	534 (61%)	340
Arthritis	28 (3%)	846

Table 21

Interval (days) between the date of onset of rash in the index case of rubella and the date of taking the swab, according to the isolation of the rubella virus

Isolation of rubella virus	Interval (days) between onset of rash and taking swab									Totals
	0	1	2	3	4	5	6	⩾7	Not Stated	
Yes	27	94	108	38	22	10	7	5	23	334
No	28	77	101	92	52	30	19	69	46	514
Percentage positive	49	55	52	29	30	25	27	7	33	39

Table 22.

Rubella haemagglutination-inhibiting antibody titres in 224 Metropolitan Police Cadets, according to area of origin

Area of origin	Rubella haemagglutination-inhibiting antibody titre *	
	< 16	≥16
London and Home Counties	5 (3%)	157
Midlands	0	10
S.W. England	1	9
N.E. England	0	7
N.W. England	0	9
E. Anglia	0	2
Scotland	1	11
Wales	0	10
Ireland	0	2

* Haemagglutination-inhibiting antibody titres expressed as the reciprocal of the highest dilution of serum producing complete inhibition of haemagglutination.

Table 23

Rubella haemagglutination-inhibiting antibody titres in 224 Metropolitan Police Cadets and the development of rubella

Number of cadets with H.A.I. titres ≥ 16 *	217 (97%)
Number of cadets with H.A.I. titres < 16 *	7 (3%)
Number of cadets who developed rubella with a rash	6
Number of cadets who developed subclinical rubella	1

* Reciprocal titres of serum dilutions

Table 24

Rubella haemagglutination-inhibiting antibody titres and dates of onset of rash in seven Metropolitan Police Cadets who did not possess antibody when in contact with cases of rubella

Case number	Haemagglutination-inhibiting antibody * titre of first serum sample (taken 25.2.68)	Haemagglutination-inhibiting antibody * titre of second serum sample	Dates of onset of rash
1	< 8	> 128	29.2.68.
2	8	> 128	29.2.68.
3	< 8	> 128	1.3.68.
4	< 8	> 128	2.3.68.
5	< 8	> 128	3.3.68.
6	< 8	> 128	3.3.68.
7	< 8	> 128	No rash

* Reciprocal titres of serum dilutions

Table 25

Frequency of rising neutralising or haemagglutination-inhibiting antibody titres in women with pre-existing antibody to rubella who were in contact with laboratory-confirmed cases of rubella

Number of contacts with antibody to rubella	Rise in titre		
	Nil	2-fold	4+ fold
84 (Pregnant)	75	9	0
22 (Non-pregnant)	17	5	0

Table 26

Rubella neutralising antibody in the control sera:

Results of tests by members of the Working Party

Serum	Laboratories						
	A	B	C	D	E	F	G
1	4	12	4	6	24	4	8
2	16	12	4	12	12	16	8
3	16	24	16	12	48	24	32
4	4	4	4	4	4	4	4
5	4	6	4	6	8	4	8
6	16	24	16	24	16	32	32
7	64	48	32	24	24	32	32
8	16	12	16	12	16	12	32
9	4	4	4	4	4	4	4

The figures are reciprocal titres of initial serum dilutions

F I G U R E S

FIGURES

Figure

1. Cases of rubella (rate per 100,000) in England and Wales during 1967 based on returns to the Royal College of General Practitioners.
2. Cases of rubella (rate per 100,000) in England and Wales during 1968 based on returns to the Royal College of General Practitioners.
3. Percentage of 2,007 women of different ages who lacked rubella neutralising antibody.
4. Immunity to rubella among 1,747 pregnant women given 750 mg. immunoglobulin and the development of infection after contact with patients who had the clinical manifestations of rubella.
5. Immunity to rubella among 75 pregnant women given 750 mg. immunoglobulin and the development of infection after home contact with confirmed cases of rubella.
6. Interval between the dates of onset of rash in the index case, inoculation with immunoglobulin and development of maternal rash.
7. Immunity to rubella among 543 uninoculated women and the development of infection after home contact with patients who had the clinical manifestations of rubella.

Figure

8. Immunity to rubella among 208 uninoculated women and the development of infection after home contact with confirmed cases of rubella.

9. Cadets who suffered from clinical rubella at the Metropolitan Police Training College before the start of the investigation.

10. Area of origin of 224 Metropolitan Police cadets in contact with rubella.

Figure 1

Cases of rubella (rate per 100,000) in England and Wales during 1967 based on returns to the Royal College of General Practitioners

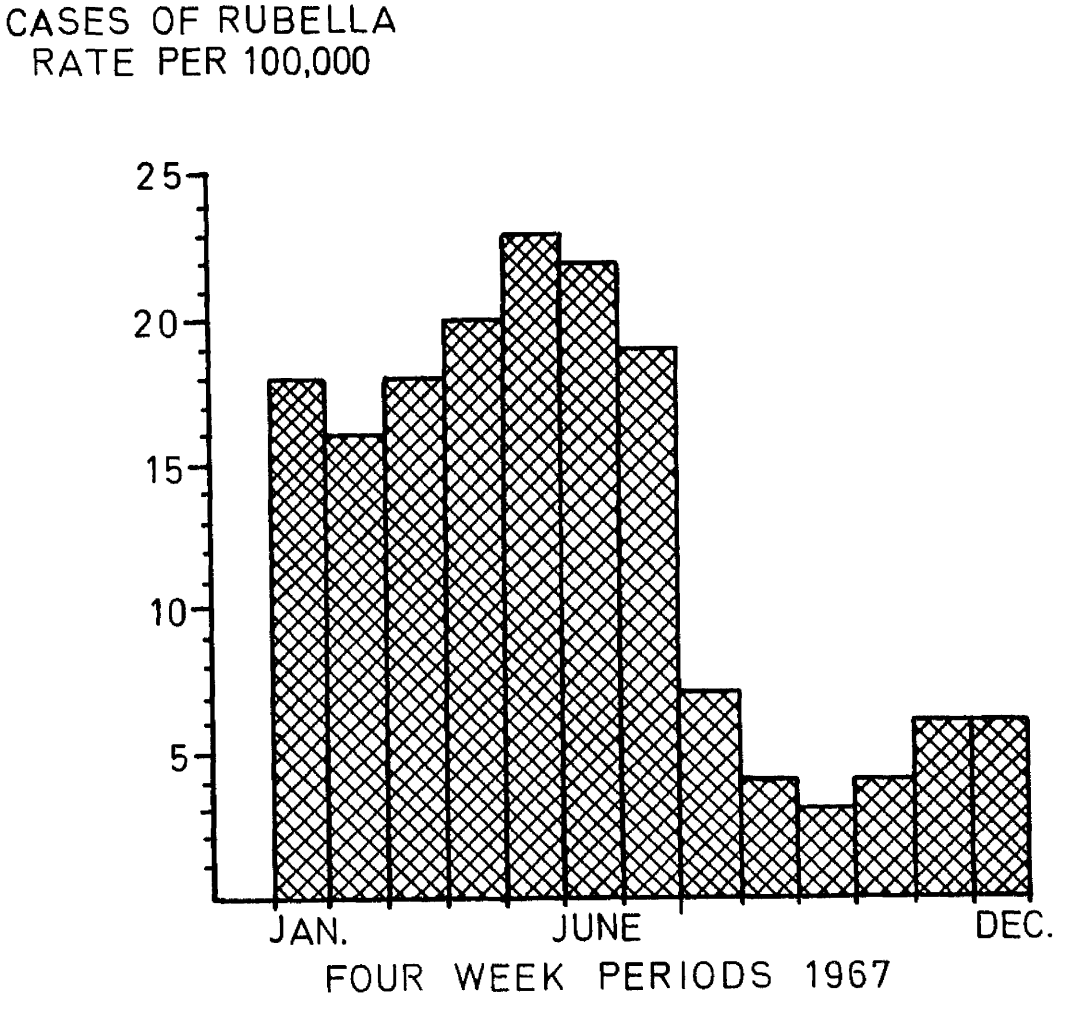


Figure 2

Cases of rubella (rate per 100,000) in England and Wales during 1968 based on returns to the Royal College of General Practitioners

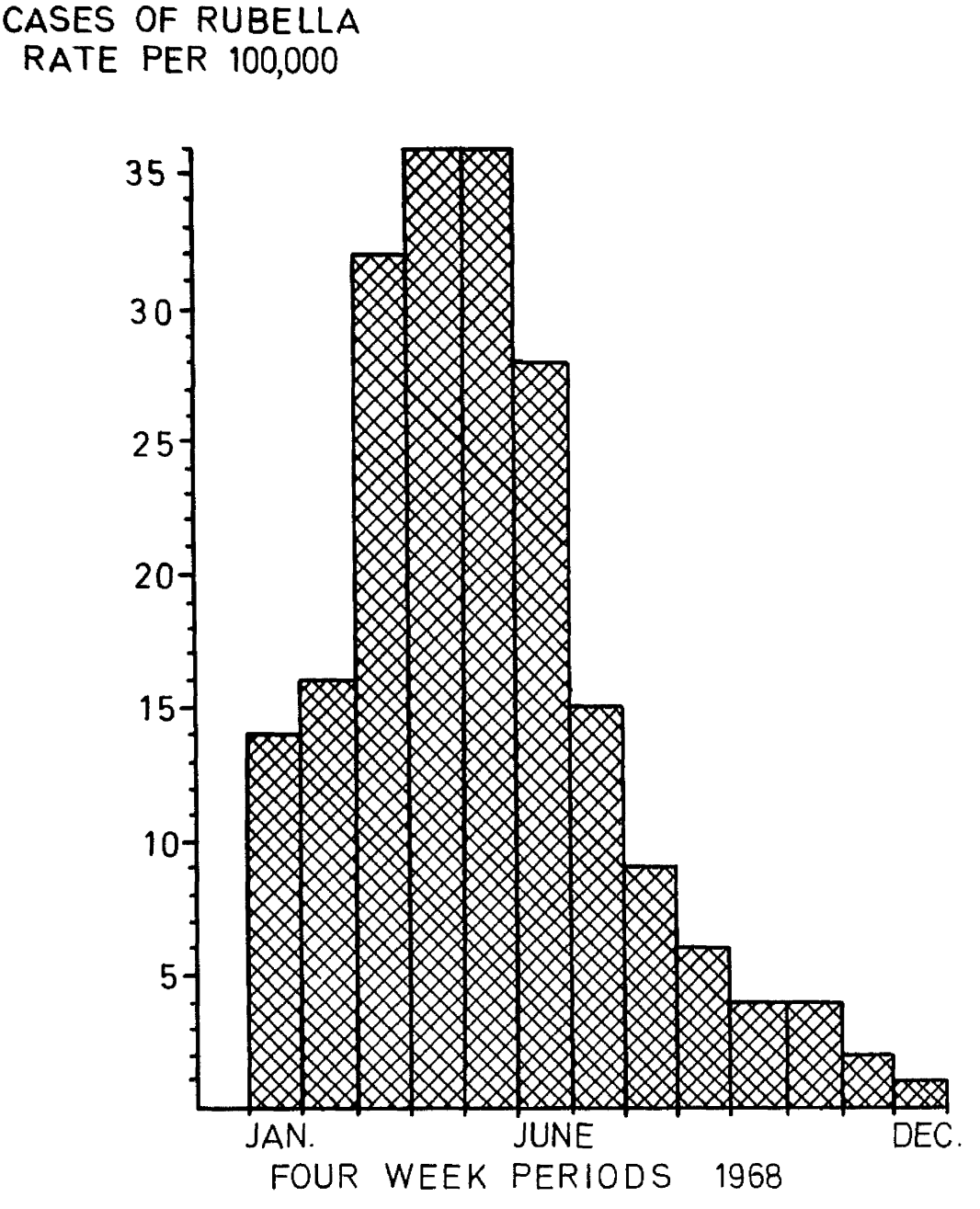


Figure 3

Percentage of 2007 women of different ages who lacked rubella neutralising antibody

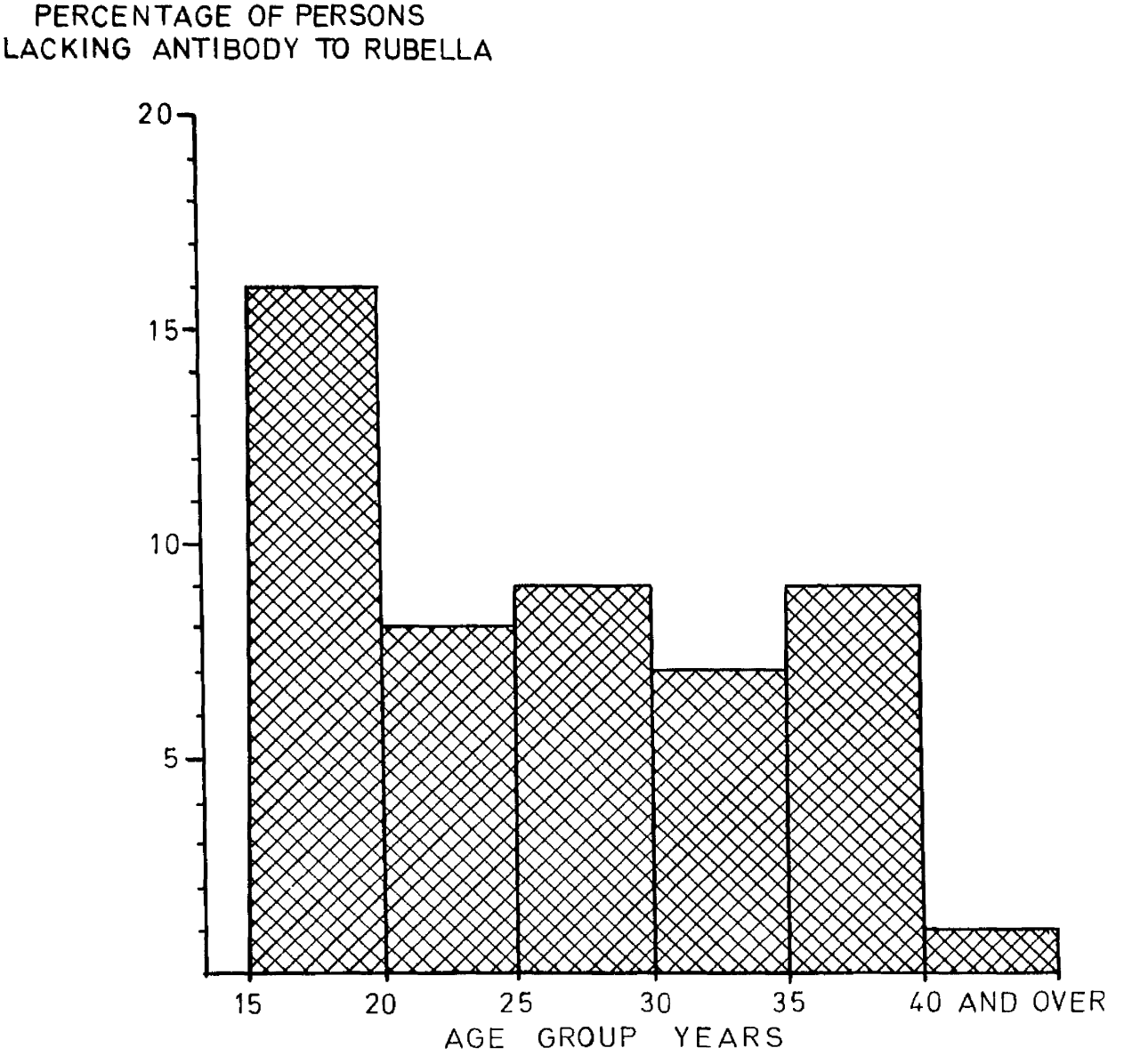


Figure 4

Immunity to rubella among 1747 pregnant women given 750 mg. immunoglobulin and the development of infection after contact with patients who had the clinical manifestations of rubella

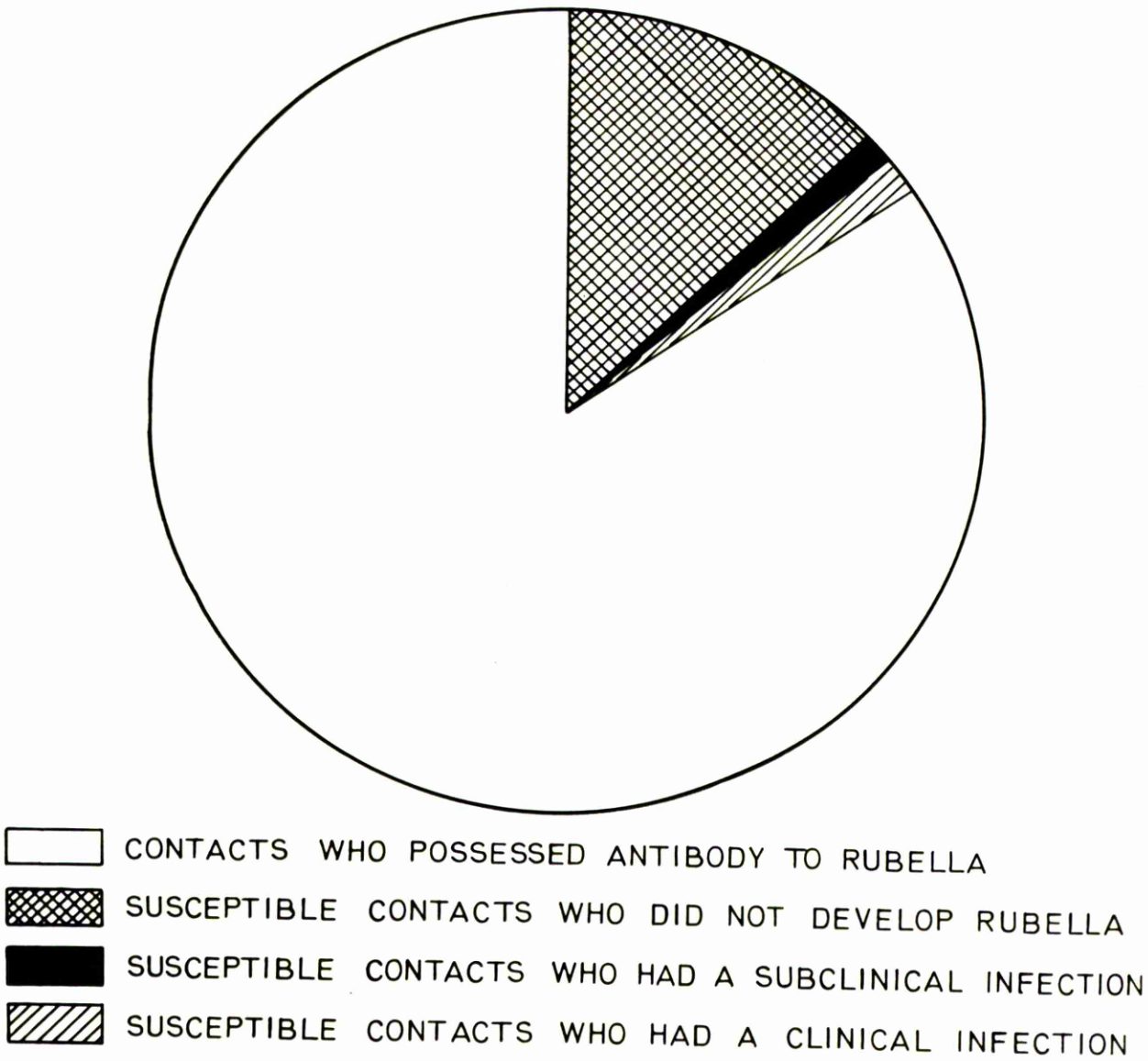
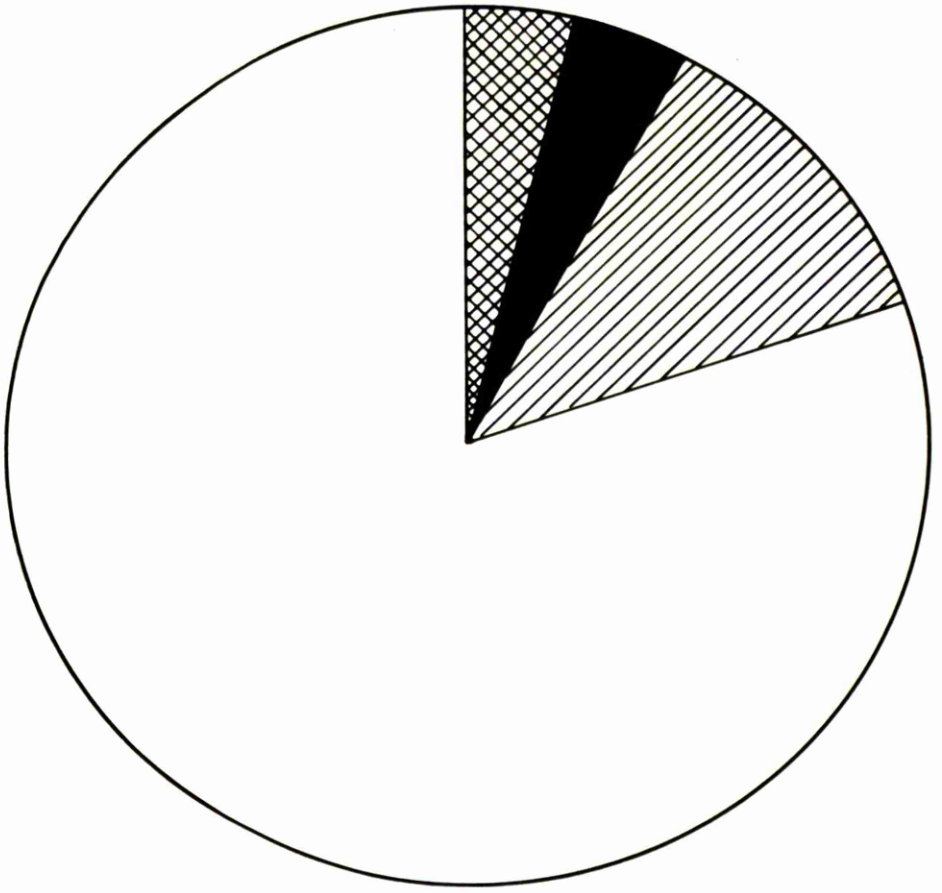


Figure 5

Immunity to rubella among 75 pregnant women given 750 mg. immunoglobulin and the development of infection after home contact with confirmed cases of rubella



CONTACTS WHO POSSESSED ANTIBODY TO RUBELLA

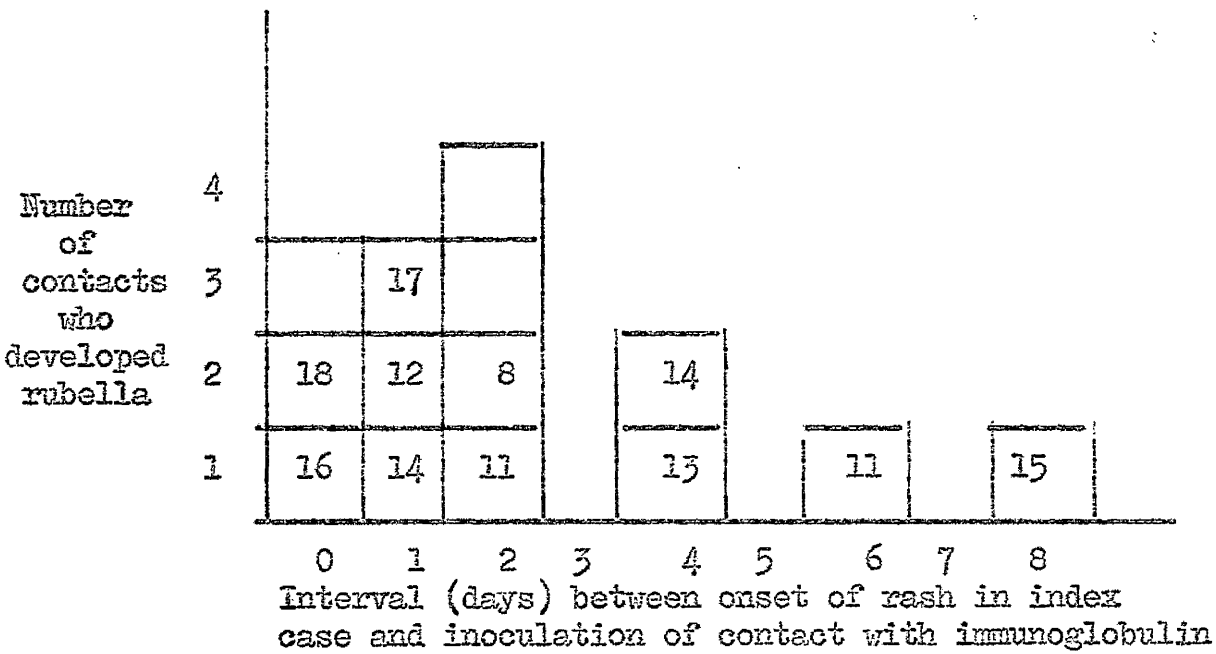
SUSCEPTIBLE CONTACTS WHO DID NOT DEVELOP RUBELLA

SUSCEPTIBLE CONTACTS WHO HAD A SUBCLINICAL INFECTION

SUSCEPTIBLE CONTACTS WHO HAD A CLINICAL INFECTION

Figure 6

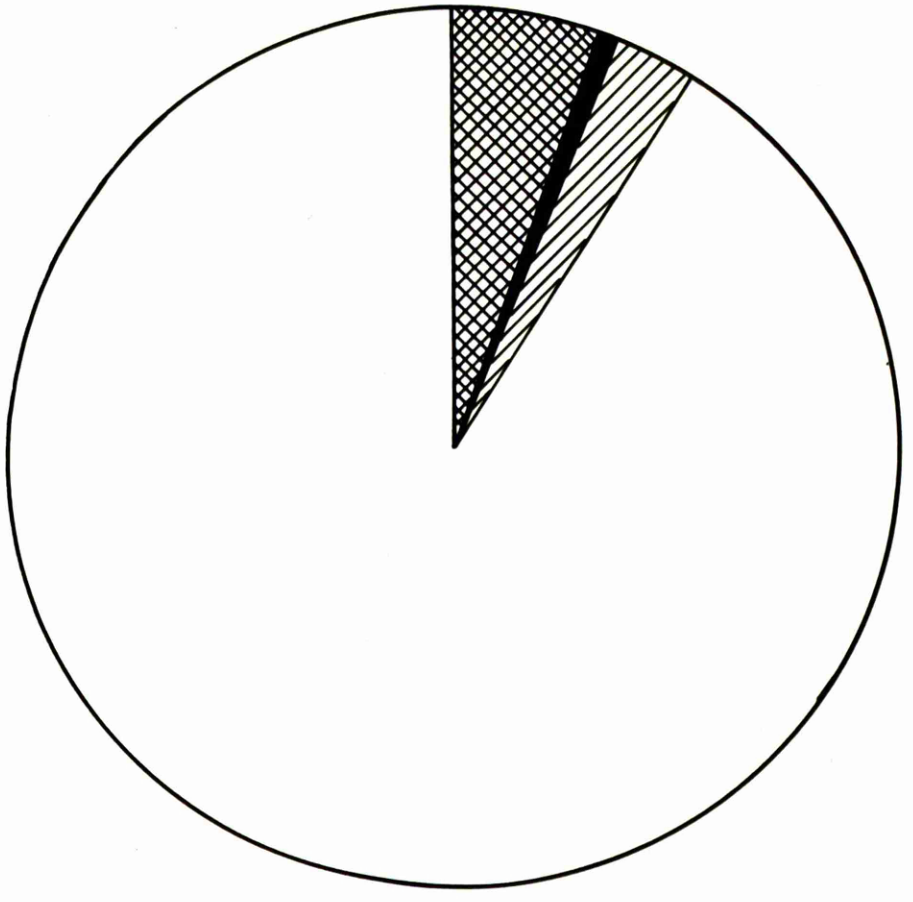
Interval between dates of onset of rash in the index case, inoculation with immunoglobulin and development of maternal rash



(Numbers in the boxes represent interval of days between inoculation and date of maternal rash; subclinical cases are shown by blank boxes)

Figure 7

Immunity to rubella among 543 uninoculated women and the development of infection after home contact with patients who had the clinical manifestations of rubella



CONTACTS WHO POSSESSED ANTIBODY TO RUBELLA

SUSCEPTIBLE CONTACTS WHO DID NOT DEVELOP RUBELLA

SUSCEPTIBLE CONTACTS WHO HAD A SUBCLINICAL INFECTION

SUSCEPTIBLE CONTACTS WHO HAD A CLINICAL INFECTION

Figure 8

Immunity to rubella among 208 uninoculated women and the development of infection after home contact with confirmed cases of rubella

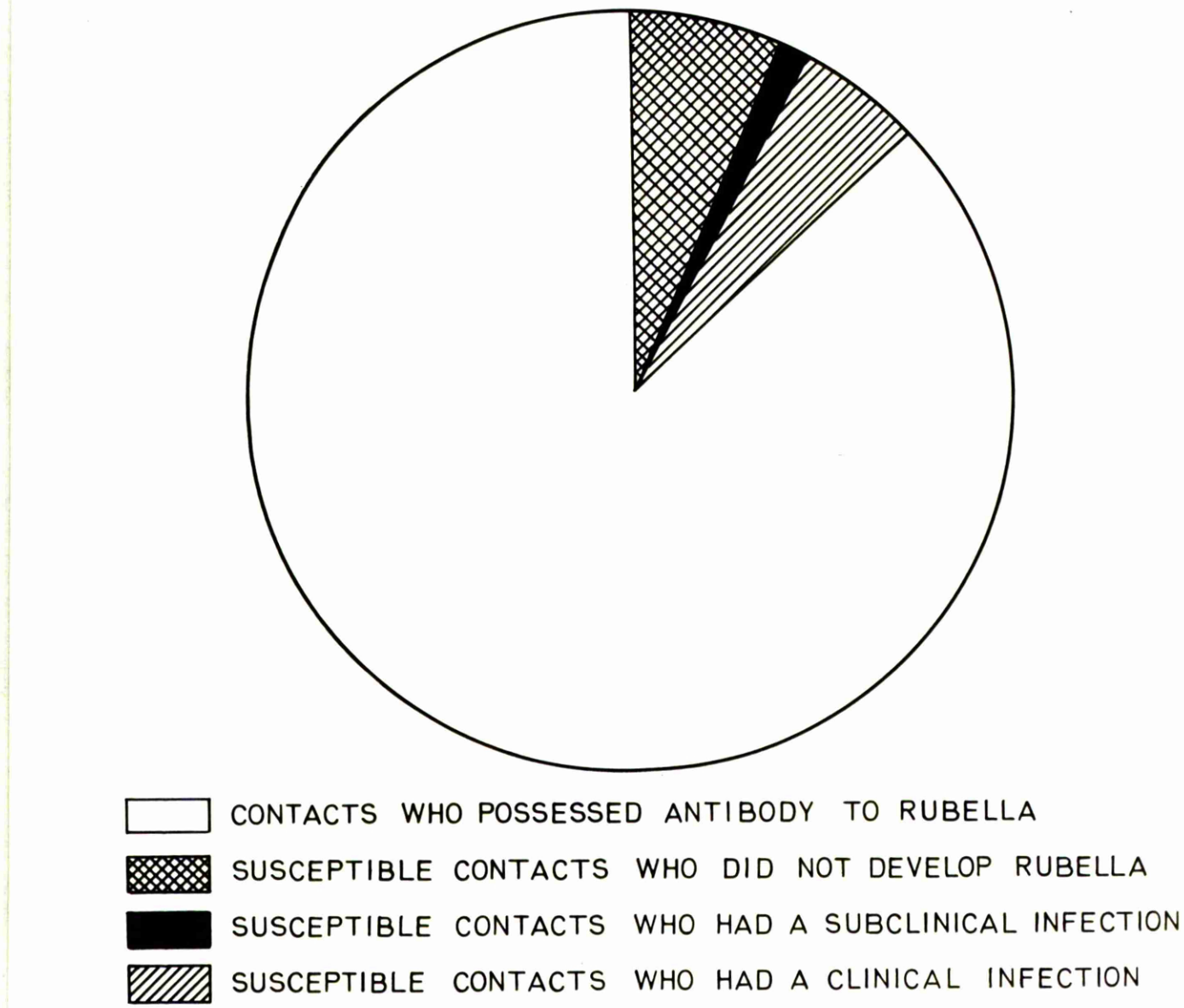


Figure 9

Cadets suffering from clinical rubella at the Metropolitan Police Training College before the start of the investigation

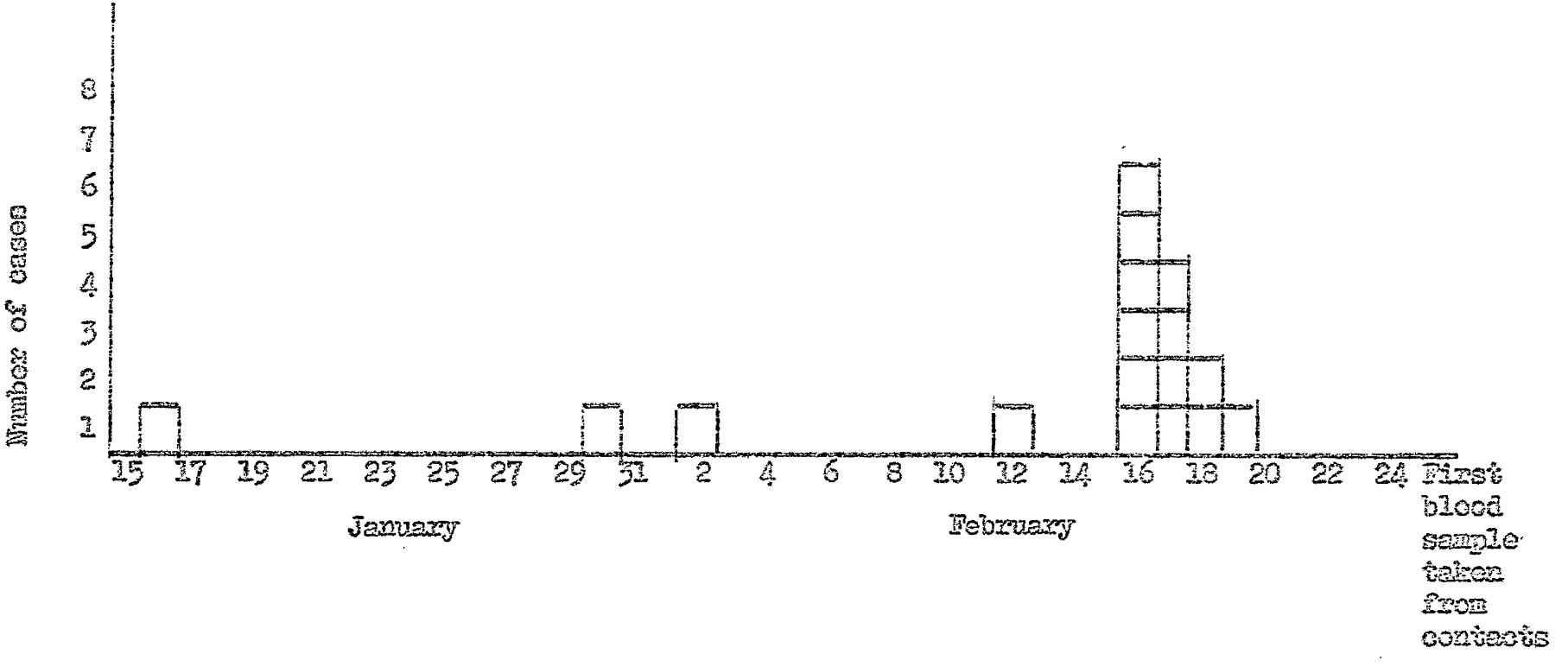


Figure 10

Area of origin of 224 Metropolitan Police cadets

in contact with rubella