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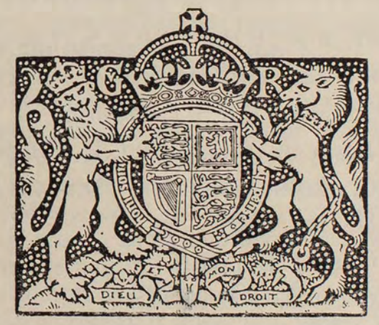
THE SOURCE OF INFECTION IN PUERPERAL FEVER DUE TO HAEMOLYTIC STREPTOCOCCI

by

DORA C. COLEBROOK



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PREFACE

Puerperal sepsis, an infective condition due to the invasion of the body by harmful micro-organisms during childbirth, is not only a heavy cause of maternal mortality but also one of the great enigmas of medical science at the present time. On the one hand, medical practitioners have for fifty years or more been living in an atmosphere permeated by teaching of the fundamental importance of anti-sepsis and asepsis. On the other hand, here is an infective state which still remains common in spite of greater precautions and better technical methods. These circumstances make the situation particularly challenging; and the challenge is not the less impressive because the condition occurs in what ought to be a normal physiological process, and often when there has in fact been little or no obstetrical interference. One thing is certain: a situation such as this must mean that knowledge of the problem of infection in general, and of this special aspect of the problem in particular, is imperfect, and that a solution can be attained only through better information about the factors at work.

It was in view of these considerations that the Medical Research Council were glad to give their support, in 1930, to the establishment of a "unit" of research in the subject at Queen Charlotte's Hospital, London, in the Bernhard Baron Memorial Research Laboratories attached to the new Isolation Block at Hammersmith. The funds which the Council were themselves able to provide for this purpose, in addition to the resources which the Hospital had available, were largely augmented by a generous subvention from the Rockefeller Foundation of New York, granted for a period of seven years. The particular inquiry here reported has also received some financial and other assistance from the Ministry of Health.

The present publication by Dr. Dora Colebrook is one of a number of valuable accounts describing the work done in the unit. Whereas it has long been known that the morbid agent responsible for most cases of puerperal sepsis is a streptococcus characterised by its ability to haemolyse red blood corpuscles, it has been realised only in the last few years that the cocci having this property in common really comprise several groups and a number of sub-groups, of which only certain members are harmful to human beings. At first sight this would appear to complicate the problem from the point of view of the investigator, but in reality it does not: indeed the recognition of these different groups and sub-groups promises to throw much light upon the aetiological problems, not only of puerperal fever, but of a wide range of infections for which haemolytic streptococci are wholly or in part responsible. In human pathology, that range includes scarlet fever, acute tonsillitis and epidemic sore throat, erysipelas, whitlow, impetigo, wound infections, "hospital sepsis", and perhaps acute rheumatism: in animal pathology, bovine mastitis, equine strangles, and other conditions.

The new technical procedures developed at the Rockefeller Institute in New York for the differentiation of the haemolytic streptococci have enabled workers at Queen Charlotte's Hospital to reach an important conclusion in respect of puerperal fever. It is that the haemolytic streptococci which are occasionally found in the genital tract of healthy parturient women are not, as was formerly supposed, identical with those causing puerperal fever, and are indeed usually harmless to their human hosts. As a corollary it would seem to follow that when the pathogenic types of haemolytic streptococci do invade the genital tract they have been conveyed to it from some outside source. That conclusion finds much indirect support from the present report.

It has been the task of Dr. Dora Colebrook to track down these outside sources in a large series of cases of puerperal fever. Her laborious work, extending over three years, is described with full detail in the following pages. Her results will be of considerable interest to bacteriologists, as showing that it is now possible, by a judicious application of various technical improvements, to obtain reliable and clear-cut results of definite epidemiological value. And to epidemiologists, as well as to obstetricians concerned with puerperal fever, the report should also be of great interest as indicating, with a very high degree of probability, what are the usual sources, outside the genital tract, from which haemolytic streptococci are conveyed to the woman in labour.

The multiplicity of these sources will at once attract attention, although it need occasion no surprise in view of the great variety of infections caused by the haemolytic streptococci. Ample confirmation of the view that the streptococci of the respiratory tract bear an intimate relation to puerperal fever is contained in Dr. Colebrook's results; but these results suggest that the respiratory tract of the mother must be taken into account as well as that of her attendants, and familial sources of infection have been incriminated in not a few instances.

The thoroughness with which this investigation has been carried out, the caution shewn in drawing conclusions, and the faithful recording of her failures as well as her successes, gives to Dr. Colebrook's report a stamp of conclusiveness absent from much earlier work on this subject.

In the nature of the case, it would be scarcely possible to obtain stronger evidence than is given by this report, and by other work from Queen Charlotte's Hospital and elsewhere, as to the responsibility of the throat and nose-carried streptococci on the one hand, and of those of various septic infections on the other, in the causation of puerperal fever. Until methods are known for ensuring a high resistance of the maternal body to these pathogenic agents, it should be recognised that it is undesirable—and indeed dangerous—for any person suffering from an acute infection of the respiratory tract to engage in maternity work. It is also unwise for nursing homes and small hospitals to deal with maternity and surgical cases under the same

roof unless adequate provision can be made for complete separation of the nursing staffs of the two departments. A wider employment of the laboratory services available throughout the country for the prompt detection of the puerperal case infected by the streptococcus, when it occurs, is to be desired.

The results and lessons of this report, if rightly applied, should achieve a reduction in the incidence of haemolytic streptococcus infection following childbirth.

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THE SOURCE OF INFECTION IN PUERPERAL FEVER DUE TO HAEMOLYTIC STREPTOCOCCI

BY

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CONTENTS

I.—INTRODUCTION

While there is general recognition of the great importance of haemolytic streptococci in the causation of puerperal fever, the actual incidence of these organisms in all cases delivered or in all cases of notifiable puerperal pyrexia is not easy to ascertain.

The following figures refer to patients sent in to hospital, admittedly the most severe cases :—

Author.	*Cases examined.*	*Infected with haemolytic streptococci.*	*Percentage mortality of cases infected with haemolytic streptococci.*	*Percentage of cases infected with haemolytic streptococci in all deaths.*
Kinloch, Smith & Stephen (1928) ..	69	61 (88%)	—	96
Smith (1931) ..	196	149 (76%)	30	93
Queen Charlotte's Hospital Clinical Report— (1931) ..	282	118 (42%)	28·8	76
(1932) ..	257	90 (36%)	21	68
(1933) ..	220	92 (42%)	21·7	71·4
Rankin (1933) ..	82	36 (44%)	37·5	81·8

A. The Source of the Infection

While clinical records show that extensive epidemics of puerperal fever occur very much less frequently than heretofore, thereby indicating that measures designed to prevent the transfer of the infecting organisms from patient to patient can be successfully employed, the greatest interest still attaches to the search for the source of infection in sporadic cases, and wherever possible for the strain which originated such small epidemics as do occur.

We have at present scanty data regarding the criteria which might enable us to assess in advance the degree of infecting capacity possessed by any given organism. It is indeed certain that the patient's chance of sickness or health in the puerperium is

determined by a balance of many factors—the invasive or other relevant properties of the microbe (Hare, 1934), the size of the infecting dose received, the mechanical factors which impede or favour entry or lodgment of the organisms, the local and general resistance of the patient, etc.

In a survey of the possible sources of infection attention is naturally directed to the organisms found in the genital tract before or during labour, in the bowel, or on the skin of the perineum of the patient, on the normal skin of the hands, in septic foci, or in the respiratory passages of either patient or contact.

Recent research, notably by Lancefield, Hare and L. Colebrook (*vide infra*), which has a very important bearing on this problem, has shown that haemolytic streptococci may be divided by biochemical and serological tests into various groups, into one of which fall practically all the strains which are pathogenic for man. To the other groups, some of which are not as yet quite clearly defined, belong those strains isolated from various situations in normal human beings and in animals.

Lancefield (1933), using the precipitin reaction with sera rich in those antibodies which are shared by members of any one of these groups of haemolytic streptococci, and by means of which differentiation may be made from the strains of other groups, found that 16 strains isolated from severe human infections, and 5 strains isolated from the contacts of persons suffering from throat infections fell into the human infective group, which she labels "group A": 18 strains from bovine mastitis fell into a "group B", and strains from the lower animals and various sources into other groups.

Reports from Queen Charlotte's laboratory on the classification of these "carrier" strains are actually in the press, or are in preparation for the press, and it would, therefore, be premature to deal with the matter here more definitely than to state that there is at present little or no evidence to show that strains which are recovered from the normal skin, including the skin of the perineum, or from the bowel are capable of initiating severe infection in human beings.

It is clear that, generally speaking, quotations of figures showing the incidence of strains of haemolytic streptococci in any particular site in the healthy human body are largely irrelevant to any discussion of their pathogenic significance, since, at present, collateral data are not available to show whether or not such strains belong to the human pathogenic group.

In respect of the strains recovered from the genital tract of women before or during labour or in the early days of the puerperium, the position is, however, clearer, as in the last few years a number of observations have been made.

Investigation of mildly infected cases by White (1927), Bryce (1931), Armstrong and Burt-White (1929) and L. Colebrook and Hare (1930) has shown that the haemolytic streptococcus is a comparatively infrequent cause of the minor febrile disturbances of the puerperium.

The results of clinical observations of the character of the puerperium of a number of women who were swabbed either before or during labour or in the early days of the puerperium are summarised in tabular form below.

The incidence of haemolytic streptococci in the genital tract before and during labour and in the early days of the puerperium

Author.	*Delivery in hospital ("H") or in the patient's home ("D").*	*Patients examined.*	*Time of examination.*	*Patients carrying haemolytic streptococci.*	*Character of the puerperium in these patients.*		
					Febrile		
					Mild infection.	*Severe infection.*	*Afebrile.*
Taylor & Wright (1930).	H.	1,123	"Before labour"	32 (2·7%)	3	—	29
Rose (1933).	D.	3,343	"Ante-natal"	80* (2·4%)	3	—	77
,,	H.	1,950	On admission	47* (2·4%)	3	—	44
,,	H.	1,950	During second stage	85* (4·3%)	3†		70
,,	H.	1,950	During puerperium	—	12†		—
Hare & L. Colebrook (1934)	D.	855	At onset of labour	13 (1·5%)	1	—	12
,,	H.	837	On 3rd or 4th day of puerperium	52 (6·2%)	11	1	40

* These strains showed beta-haemolysis on the surface of blood-agar plates, but the soluble haemolysin test (p. 41) was not performed with them.
† The severity of the infection was not specified.

The collected clinical evidence shows that the haemolytic streptococci present in the vagina before labour, and the great majority of those present after delivery, are relatively harmless to the parturient woman. An explanation of this fact is given by the work of Lancefield (1933), Hare and L. Colebrook (1934) and of Lancefield and Hare (1935).

The biochemical tests of Hare and L. Colebrook (1934), amplified by the serological tests of Lancefield and Hare* (1935), gave the following results:—

(1) 855 *women were swabbed at the onset of labour:* from 13 of these women strains of haemolytic streptococci were

* The figures of Lancefield and Hare in this paper also include 34 strains of streptococci which produced an area of beta-haemolysis on the blood agar plate, but which did not give the soluble haemolysin test.

isolated, none of which was of the human pathogenic type : 12 of these women had an afebrile puerperium, one woman had a definite but not severe infection.

(2) 837 *women were swabbed on the 3rd or 4th day of the puerperium :* 52 strains of haemolytic streptococci were recovered : the strains isolated from 50 women were not of the human pathogenic type : 39 of these 50 women were afebrile in the puerperium, 11 showed a mild infection : the strains isolated from 2 of the 52 women were of the human pathogenic type : one of these women showed a severe infection, the puerperium of the other was afebrile.

(3) Of the uterine strains isolated from 46 *severely infected women* 45 were of the human pathogenic type, while one was not of this type, but this woman was suffering from a concurrent staphylococcal septicaemia.

Septic conditions of the skin remote from the genital tract.—No emphasis need be laid on the grave risk of direct spread to the genital tract from septic foci on the hands of the attendants. Less attention has been given to the danger arising from sources of infection on the skin of the mother or a member of her household. Smith (1931 and 1933), investigating the source of the infection of 49 patients, isolated a strain identical with the infecting strain from a sore on the hand of the doctor once, and once on the hand of the patient. Two such instances will be described in this series.

The respiratory tract.—Although the human nose and throat are known to be a prolific source of haemolytic streptococci, and although the harmful potentialities of these organisms are fully appreciated in surgery, there is by no means such unanimous recognition of their significance in midwifery.

Systematic swabbings have shown that from 5 to 30 per cent. of healthy persons may be carriers of haemolytic streptococci at any time. It is not proposed, however, to submit a review of the available statistical evidence regarding the carrier rate ; such data are not relevant to the purpose of this inquiry, since there is no published evidence to show the proportion of strains belonging to the human pathogenic group—the only group with which this study is concerned—which are to be found among all carrier strains. It is reasonable to suppose that the incidence of organisms belonging to the human pathogenic group will be higher in institutions or households where persons are in contact with cases of streptococcal infection.

It may, however, be said that the available evidence to date all goes to show that the human respiratory passages constitute the only important stronghold of those strains of haemolytic streptococci which are capable of initiating severe puerperal infection.

A summary is given by Smith (1931 and 1933) of the direct and indirect evidence which in recent years has accumulated to incriminate these organisms of the respiratory tract. The studies of Smith himself constitute an important link in the chain of direct evidence. Using the technique of reciprocal absorption of agglutinins, he was

able to show that in the case of 39 out of a total of 49 patients a strain identical with the infecting strain was found in the respiratory tract of patient and/or contact (Table V). From a survey of his data Smith claims that in those groups he had found *the actual source of the infection.*

B. Objects of the Present Research

From the technical, as well as the epidemiological, standpoint, it was felt that confirmation of these findings would be of value. In reporting his technical results Smith gives no hint of the difficulties and inconsistencies which have often proved so serious an obstacle to the successful classification of haemolytic streptococci. In an earlier paper (1927) he describes such happenings and notes that Griffith (1926) and James (1926) had met with similar experiences. Griffith (1926 and 1927), using direct (slide) agglutination and absorption of agglutinins, found the methods reliable and useful for the detection of the dominant antigen in strains of haemolytic streptococci ; he gives warning against the use of disproportionately heavy absorbing doses and discusses the meaning of anomalous results. More recently Andrewes and Christie (1932) in their attempt to " bring order out of chaos " were largely defeated by the inconstant behaviour of their absorbing strains and by the " non-specific " qualities of their sera. They conclude that these organisms are " in a state of constant flux in which it is difficult to find any foundation for a permanent systematic classification ".

This study was planned *primarily* to investigate these two apparently diametrically opposed experiences : to determine whether the method of absorption of agglutinins can be relied on, as in the case of other micro-organisms, for the differentiation of definite and stable serological types among the haemolytic streptococci giving rise to puerperal infection ; whether by following the general technique of Smith—modified in certain respects for reasons to be explained—it is possible to obtain equally clear-cut results with equal regularity ; or whether the application of the method to these organisms is strictly limited either by technical difficulties or, as in the experience of Andrewes and Christie, by its liability to show results which are open to fallacious interpretation ; and whether these limitations can in any way be avoided or overcome.

The second objective was to apply the technique to extend the existing knowledge of the epidemiological importance of the respiratory tract strains isolated from patients and the contacts of patients suffering from the more severe forms of puerperal haemolytic streptococcal infection.

The third objective was to determine the number and frequency of appearance of the serological types described by Griffith (1935) among the strains isolated from a series of cases of definite infection.

Further, it is hoped that the technical experience gained may eventually be applicable to other problems of infection by these organisms.

Opportunity was afforded by Dr. F. Griffith's generous gift of his type strains and their corresponding sera to investigate whether direct (slide) agglutination with these absorbed sera and the technique of absorption of agglutinins of sera prepared from strains of the patient-contact groups gave concordant results, and whether discordant results, if any, were to be attributed to faulty technique, to the fact that certain of the type sera are imperfectly exhausted of their " group " antibody, or to erratic behaviour of the absorbing strains or of the sera under absorption.

II.—GENERAL DESCRIPTION OF THE SEROLOGICAL METHODS USED

The serological relationship of the strains of the patient-contact groups was studied by the techniques of direct agglutination and absorption of agglutinins. The sera used for these tests were :—

(*a*) Absorbed, type-specific sera prepared by Dr. F. Griffith from strains of different serological type isolated by him from various human infections ; these sera were used in direct agglutination tests on the slide.

(*b*) Sera prepared in the course of this study from the infecting strains isolated from the patients and from certain of the extra-genital strains of patients and their contacts ; the tests with these sera were carried out in the water-bath. The degree of absorption of the serum effected by the homologous strain, by the other strains of the patient-contact group and by certain control strains was determined ; and in many cases the results obtained in the absorption experiments were compared with those of direct agglutination by unabsorbed samples of these same sera of suspensions of the strains used for their absorption.

The strains of many of the groups were tested both with the sera of known type (under (*a*) above) and with the sera prepared from a strain or strains of the patient-contact groups (under (*b*)).

A. Direct (Slide) Agglutination with Absorbed Sera

This method of identification of strains has the great advantage that the actual test on the slide demands the minimum expenditure of time and material, and, when a clear-cut reaction is obtained, a verdict on the identity of strains may be given with the minimum of delay. A negative result in one or two tests is without significance, however, on account of the tendency shown by suspensions of strains to vary in agglutinability.

B. Absorption of Agglutinins

The results of this method are generally accepted as the final criterion of antigenic relationship between strains. With these organisms the chief technical difficulties to be overcome are in the preparation of sensitive and stable agglutinating suspensions (p. 46),

and of rabbit sera of sufficiently good quality (p. 60), and in the necessity for the use, in the case of certain sera, of very heavy absorbing doses.

The chief sources of error lie in the liability of certain anti-streptococcal sera to lose their homologous agglutinins when treated with heterologous strains, and of different suspensions of an absorbing strain to give inconsistent results with a serum. In a later section of the text are described experiments with five sera (p. 55) which showed the former and with 4 sera (p. 59) which exemplified the latter type of irregular result. Minor degrees of absorption by heterologous strains (p. 54) were not infrequently met with.

Individual sera were found to differ greatly from each other in the size of their " minimum absorbing dose " (" M.A.D."), that is, the smallest number of cocci of the homologous strain which is able to effect complete absorption of the serum.

" *Complete absorption* " *of a serum.*—The standard aimed at was a degree of absorption not less than 96·8 per cent. Experiments in which this standard was not attained and a 93·75 per cent. value for " complete absorption " was accepted are shown in Table I.

In the earlier part of this work, following the most recent methods of Smith (1931, 1933 and personal communication), the absorbing dose used per c.c. of a 1/50 dilution of serum was the cocci yielded by 100 c.c. of 0·2 per cent. glucose phosphate broth. Since the total yield of different strains of cocci in different batches of broth has been found to vary between 20,000 million and 200,000 million per 100 c.c., it is clear that a serum whose M.A.D. was 5,000 million per c.c., treated with the deposit from 100 c.c. of a very profusely growing culture, would receive, perhaps, 40 times the M.A.D., while another serum whose M.A.D. was 60,000 million per c.c. would be incompletely absorbed by the deposit from a poorly growing culture. And in any one absorption experiment a sample of a serum treated with one strain might receive 10 or more times the dose received by a sample treated with another strain.

Further, the practice of carrying out the absorption experiments at a dilution of the serum fixed without regard to the titre emphasises the disparity between the absorbing doses. For, if two sera whose titres are, respectively, 25,600 and 1,600, equally amenable to absorption by their homologous strains at a dilution of, say, 1/32nd-of-the-titre, are both absorbed at a 1/50 dilution, the dose of cocci just sufficient completely to absorb the former will be very much more than sufficient to absorb the latter.

It seemed not unreasonable to fear that the use of indiscriminate absorbing doses whose size and relation to the M.A.D. is unknown may obscure the antigenic differences between strains and lead to false epidemiological conclusions. At the same time it was recognised that in any absorption experiment two serologically identical strains may exhibit some relatively slight degree of difference in their absorbing power, and that, in order to effect absorption of the last traces of agglutinin it may be necessary to use up to two

or even three times more cocci of the one strain than of the other. There would be nothing surprising in this since a relatively much larger dose is needed to take out the last traces of agglutinin from a serum than to take out, say, the first 50 per cent. (Eisenberg & Volk, 1902). Moreover, admittedly, the relatively crude " opacity " method gives only a rough comparative estimate of the number of cocci.

The experiments were planned so that the dilution of the serum in the absorbing mixture should be a constant fraction, namely, 1/32nd, of the titre of the serum. Circumstances which led to the use of a 1/16th or a 1/64th-of-the-titre dilution are described on pp. 49 and 51, under the description of the technique.

The arbitrary criterion adopted in formulating a standard of antigenic identity was that the absorbing dose at the given dilution should not be greater than three times the M.A.D.

The selection of " control " strains.—The drawing of false conclusions from experiments in which the homologous agglutinins are absorbed by heterologous strains can and must be guarded against by the inclusion, in every experiment with unknown strains, of " control " strains known to be serologically different from each other and, when its type is known, from the homologous strain. When the type of the homologous strain is not known, with a limited range of strains to draw from, there is just a chance that one of them may prove to be serologically identical with the homologous strain. In this case a reciprocal absorption experiment will confirm the evidence of the first experiment.

This point is particularly stressed because in the description of the experiments conducted by Smith (*loc. cit.*) there is no mention of this danger and in the case of more than 1/3rd of his experiments deductions as to the identity of strains appear to have been made from experiments in which all the strains tested absorbed completely. The inclusion in these experiments of a strain or strains which failed to absorb the agglutinins would have served to make the results more convincing.

When they were available, strains presumed to be identical with the homologous strain, e.g., strains isolated from another site in the patient (the blood, the peritoneum, etc.) and/or the infecting strains of other groups known to belong to the same serological type as the homologous strain, were included in the experiment.

The results recorded in this study are based on experiments in which absorption has been carried out with a serum dilution of 1/32nd, or exceptionally, 1/16th of the titre ; in which " complete absorption " by the homologous strain at the M.A.D. and by other strains shown by collateral evidence to be identical with the homologous strain, at a dose not more than three times as great as the M.A.D., is contrasted with " no absorption " (p. 50) by heterologous strains.

The titrations of the treated samples of serum may be carried out on the slide or in the water-bath. The latter alternative is

preferred here on the ground that the experimental conditions are more constant and more controllable, and that it permits of the almost simultaneous reading of the titrations of all the samples of the serum, and, consequently, of a roughly comparative estimate of the degrees of absorption effected by the various absorbing strains.

C. Direct (Water-Bath) Agglutination with Unabsorbed Sera

Cross agglutination of heterologous strains may occur frequently with low dilutions of unabsorbed sera. Preliminary tests showed that the sera prepared in this work when used at a sufficiently high dilution, which is a constant fraction (1/32nd) of the titre, for the most part agglutinate strongly the strains serologically identical with the homologous strain, and fail to react or react weakly with suspensions of strains antigenically different from these.

III.—THE EPIDEMIOLOGICAL RESULTS

Since, from a review of the epidemiological findings, which are in general agreement with those of Smith (1931, 1933) and others, certain definite facts emerge which have an important bearing on the practical problems of maternal mortality due to infection, and which, it is hoped, will be of interest to practitioners of midwifery and to public health administrators, the next sections are devoted to a detailed description of these findings and to a discussion of the significance of the knowledge gained.

The technical methods used and the technical results on which these epidemiological conclusions are based are fully described in the later sections.

A. The Collection of the Material

The material was derived from cases and contacts of cases of puerperal fever admitted to the Isolation Block of Queen Charlotte's Hospital through the agency of medical officers of health, private doctors and the officers of institutions ; and also, for about half the period covered, from cases occurring throughout the country, by arrangements with the Ministry of Health.

By means of a simple form issued to the medical officer in charge of the case, the names of the attendants and the part they played in the confinement and puerperium are ascertained and relevant facts pointing to the desirability of swabbing members of the household of the patient are elicited, special attention being paid to a history of recent acute or chronic infection of the nose or throat or to any other focus of sepsis.

The nose (anterior nares), throat (tonsils) and, in certain cases, gums of the patient are swabbed and every effort is made to secure the full complement of swabs from the contacts.

The control over material received from patients and the contacts of patients not admitted to this hospital was very limited, with the result that the number of swabs was usually incomplete ; in the case of 6 patient-contact groups *cultures* of the organisms were

supplied in place of actual swabs; and for some of the cases only the scantiest data were available. The significance for the results of deficiencies of the material and of "negative" swabs are discussed in due course (p. 32).

For the most part, the uterine infection of the cases studied was a severe one, and, in a certain number of cases, the groups were selected on account of some point of epidemiological importance or interest to the medical practitioner or the family of the patient, such as the occurrence of a recent case of scarlet fever or of tonsillitis, or the existence of chronic nasal discharge in an attendant or a member of the household of the patient: and, other things being equal, preference was given to those groups in which swabs from the respiratory tract yielded an abundant growth of streptococci. In many cases patient and contacts were not swabbed until some days after the onset of the fever. Material was not collected, however, when the onset of pyrexia was later than two or three weeks after delivery.

The groups selected for study are thus just those in which there was the greatest chance that strains identical with the infecting strain would be found in the nose or throat of patient or contact.

It is recognised that from such small numbers as those submitted dealing with material collected under most variable conditions, very imperfectly controlled by the laboratory staff, only general deductions can be made.

Although, then, strictly speaking, the results of this study furnish direct statistical evidence to show how high is the degree of probability that in this type of case and in the circumstances described only, the streptococci which are found in the respiratory tract of the patient and of the persons in her immediate environment will be found to be serologically identical with her infecting strain, yet they are, presumably, applicable at least to all cases of this infection admitted to hospital, since it seems reasonable to believe that a sufficiently comprehensive search would reveal a source for the infection in these cases.

Direct statistical evidence of the risk to parturient women in general which the presence of haemolytic streptococci of the human pathogenic type (p. 8) in the respiratory tract entails could only be obtained by a study of the post-partum history of all women who, themselves, or whose contacts, were known to be carriers of these organisms at the time of, or shortly after, labour. The accumulation of such evidence would involve inquiry on a very large scale, with ante- and post-natal swabbings of the genital and respiratory tracts of the patient and the respiratory tracts of her attendants and the members of her household, conducted with a most stringent system of control over the collection of swabs and data. A preliminary experiment to explore the possibilities and limitations of such an inquiry was conducted in this laboratory in 1933 by Dr. R. M. Fry (*see* Hare and L. Colebrook, 1934). For a period of 12 months an attempt was made to secure swabs from the vagina, the nose and

the throat of every woman on the Queen Charlotte's district at the beginning of labour. The swabs were taken by the nurse directly she arrived at the house for the confinement, that is, before there had been any appreciable opportunity for any organisms which might be present to pass from one person or one site to another. From the 855 vaginal swabs 13 strains of haemolytic streptococci were recovered, none of which had the character of the human pathogenic type of streptococcus. One only of these women who was carrying a haemolytic streptococcus in her vagina had a febrile puerperium and in her case it so happened that ante-natal nose and throat swabs had not been secured.

B. Criteria for Determining the Source of Infection

While, then, the evidence as to the aetiological significance of these organisms in the respiratory tract is accepted in general, it must be admitted that in many cases clear evidence to incriminate any particular strain among those isolated is lacking.

The experimental evidence of this study shows that in the case of approximately 1 in every 3·5 of the patients for whose infection a possible source was found in the respiratory tract, the extra-genital strain identical with the infecting strain was found in both patient and contact and that not infrequently a number of contacts of a patient were carriers of strains identical with the infecting strain. The demonstration of these multiple strains is in keeping with all the facts that are known regarding the facility with which these organisms are acquired, and serves to emphasise the need for caution in the attempt to draw any picture of the exact epidemiological sequence of events. A delay of several days, such as may occur between the onset of the fever and the taking of the swabs, gives ample opportunity for the dissemination of strains from the respiratory tract of one person to another or from the infected discharges of the patient. When, as not infrequently happens, the examination of material from an institution in which a case of puerperal pyrexia has occurred, reveals strains of the same serological type as the infecting strain in the noses and throats of a number of the staff, many of whom, indeed, may not be direct contacts of the patient, detection of the original source of the infection becomes quite impossible. In two instances in this series strains identical with the uterine strain of an infected patient were isolated from the throats of a number of uninfected lying-in patients in the maternity department of an institution.

And even when the finding of no more than one throat or nose strain in an attendant strongly suggests a causal relationship between this strain and the infection, consideration must be given to the possibility that the patient may have contracted her infection from an unsuspected source without carrying the strain in her throat and that the attendant may have picked up this strain either from the same source as the patient, or from the patient after the onset of the fever.

The likelihood that identical strains found in the uterus of the patient and the respiratory tract of an attendant had been acquired independently is very remote when the strain in question is a type rarely met with, but less remote when it is a type which is prevalent in the throats and noses of the general community.

For these reasons it is felt that until some more exact epidemiological methods can be applied to this problem, and more reliance placed in the technique of swabbing (p. 33), the designation "a possible source of infection" must always be preferred to "a source of infection."

C. Classification of Epidemiological Facts relating to the Patient-Contact Groups Investigated

In this section is given a short description of the relevant epidemiological facts concerning each patient-contact group investigated, the conditions in which the confinement took place and the results of the search, in patient and contacts, for a strain identical with the infecting strain. It has been thought justifiable to hazard an opinion on the most probable origin of the infection, but due consideration has been given to the fact that the course of events is in many cases obscured not only by lack of direct evidence as to the exact mode of transference (p. 34) of these organisms to the genital tract, but also by frequent failure to secure adequate data and the full complement of nose and throat swabs from patients and contacts of many patient-contact groups.

A study of the obstetrical history of the cases has not come within the scope of this inquiry.

1. Patients for whose Infection a Possible Source was found in both Patient and Contact *

(*a*) *Patients Confined in an Institution*

(1) *The patient, P*1 (*CUR* 9),* *p.* 25.—The material of this group was derived from three small outbreaks of streptococcal infection in a general hospital.

(i) A patient, P5, in the surgical block died of acute streptococcal peritonitis after a clean operation. From the peritoneal fluid and from the throat of the theatre sister identical strains of haemolytic streptococci were recovered in almost pure culture. From the throat of the surgeon a strain of different type was isolated.

(ii) About 6 weeks later *cultures* were received in this laboratory of strains isolated from 2 patients and from 5 members of the staff of the maternity department. The uterine strains of the 2 patients and the throat strains of 2 uninfected patients and of 2 nurses were shown to be identical with each other and with the strains isolated from the surgical patient and the theatre sister.

* In certain of the following groups there were two or more patients (P1, P2 and P3) of whom, perhaps, one was carrying a strain identical with the infecting strain in her nose or throat, while the nose and throat swabs of the other patient or patients were negative for haemolytic streptococci. These patients and their groups therefore appear under different headings: an asterisk and a page number following the designation of the group shows that this is so.

(iii) Five weeks later 3 puerperal patients became infected. Haemolytic streptococci were isolated from 3 of 5 nurses swabbed. Strains isolated from the uteri of 2 patients, the throat of one (P1), the throat of a patient whose puerperium had been afebrile, but who developed a sore throat (and subsequently scarlet fever) on the day of her discharge, and from the throats of 3 nurses, were found to be identical with each other and with the infecting strains of the patients and the throat strains mentioned under (*a*) and (*b*) above. Nose swabs of the persons in this group were not examined.

(2) *The patient* (*HAY* 13).—This patient was delivered by a midwife who was suffering from a chronic infection of the antrum, and who, it was found, had been associated with various sporadic streptococcal infections occurring outside the maternity department. From her nose and throat and from the nose of the patient strains identical with the infecting strain were isolated. Swabs from two other nurses in the home showed no streptococci.

(3) *The patient* (*WAR* 32).—Three patients contracted puerperal fever in a general hospital in which sporadic cases of scarlet fever had occurred shortly before. Two of these patients, who were convalescent and showed no haemolytic streptococci in their genital tracts at the time of the infection of the third patient, had been transferred to a fever hospital when they developed scarlatiniform rashes. The staff and patients in the department were all examined. As there was a temporary shortage of blood plates in this laboratory at the time, the nose and throat swabs of each individual were smeared on to one plate. Haemolytic streptococci—which were counted as throat strains—were recovered from 4 of 13 doctors and nurses and 2 of 7 afebrile patients swabbed. Complete tests were carried out with the throat strains of the infected patient and with one only of the contact strains: these strains were identical with the infecting strain: the other strains appeared to be not of this type.

In the case of these 3 patients a study of the epidemiological evidence leaves no doubt that the contact strain was responsible for the infection.

(4) *The patient P*3 (*HAR* 12) * *p.* 23.—This patient, whose infecting strain was identical with those of the two patients referred to under 3 (p. 23), was not known to have been attended by either of the two nurses from whose throats strains identical with the infecting strains were isolated. This fact would suggest that an undiscovered carrier or some contamination of utensils, etc., was responsible for her infection.

(5) *The patient* (*POP* 22).—In the nursing home in which this patient was confined, 3 babies died of acute peritonitis in the 3 weeks following the date of the onset of her pyrexia. The peritoneal fluid of the first baby was not examined. From the nose and throat of the patient, from the throats of the doctor and nurse who attended her and from the peritoneal fluid of the other two babies, strains identical with the uterine strain of the patient were recovered. In the home at the time another patient developed tonsillitis, and a nurse was away with a septic finger: neither of these two persons was swabbed. The patient's own baby was healthy.

Swabs from the remaining 3 members of the staff of the home showed no haemolytic streptococci.

(6) *The patient* (TRI 30).—In a nursing home strains of haemolytic streptococci were recovered from both the nose and throat of the infected patient, of the doctor and of a nurse in attendance, and from the throat only of another nurse and a maid. Swabs from 3 other nurses and a doctor who anaesthetised the patient were negative for haemolytic streptococci. All the strains isolated from the patient, the nurses and the maid were identical with the infecting strain. The doctor's throat strain was not of this type, and as so many other possible sources of infection had been found his nose strain was not tested.

(7) *The patient* (*LEC* 15).—This patient and another patient (WIN 33a) suffering from peritonitis were admitted on the same day from a nursing home) The infecting strains were not of the same serological type. From the nose of the patient LEC, and from the throat of a nurse who attended her, strains identical with the infecting strain were isolated. The swabs of 4 other nurses and a doctor showed no haemolytic streptococci. A nurse who, it is said, attended the patient WIN only during the puerperium, had recently suffered from attacks of sore throat, and was under suspicion of having been responsible for 3 cases of tonsillitis in infants under her care, was not swabbed as she had already undergone a tonsillectomy in another hospital. For this reason the source of the infection of the patient WIN was not investigated. It is not unreasonable to conjecture that this nurse may have been carrying a strain of different type from that of the nurse who attended the patient LEC and that this strain may have been responsible for the infection of the patient WIN.

(8) *The patient* (*TEV* 28*a*).—From the swabs of 5 nurse-contacts of this patient 1 strain of haemolytic streptococci was recovered : this strain, and the strain found in the nose of the patient, were identical with the infecting strain.

The fact that these 5 patients were confined under institutional conditions would appear greatly to favour the probability that they did not infect themselves from their extra-genital strains.

(*b*) *Patients Confined in their own Homes*

(1) *The patients P*1 *and P*2 (*BUC* 4*a*), * *p.* 24.—The patient, P1, delivered by a midwife, who had suffered from tonsillitis 3 months earlier, became infected. Two months later, another patient, P2, of this midwife, was admitted to this hospital : both women developed peritonitis. Their infecting strains were identical with each other, with strains isolated on the two occasions from the nose of the midwife, and with their own throat strains. A week later, another woman, P3, whose nose and throat swabs showed no haemolytic streptococci, was delivered by this midwife and became febrile : the infecting strain was identical with the infecting strains of the other 2 women ; on this occasion no strain of haemolytic streptococci was recovered from the nose or throat of the midwife but a strain of the same type as the infecting strain was isolated from the throat of another attendant of the third patient. The husband of the second patient developed tonsillitis 5 days after his wife's confinement. Strains recovered from his nose and throat were identical with the infecting strains.

(2) *The patient* (*HIG* 43).—Strains of haemolytic streptococci were isolated from the throats of the 4 children of this patient, one of whom had suffered from otitis media shortly before the confinement. The throat strains of this and another child and the nose and throat strains of the patient were identical with the infecting strain. The strain isolated from the husband was not of this type : the other 2 strains were not tested. The swabs of the nurse gave no haemolytic streptococci.

(3) *The patient* (*PIC* 21).—The midwife who delivered this patient had had 3 attacks of tonsillitis in the 2 months preceding the delivery. Strains identical with the infecting strain were found in her throat and in the nose and throat of the patient. The nose swab of the midwife was missing. A nurse who attended this patient after she became febrile contracted a septic hand due to a streptococcus of the same type as the infecting strain.

(4) *The patient* (*RIC* 51).—The husband of this patient developed " influenza and sore throat " 14 days before the confinement. The patient was admitted to hospital, in labour, with a temperature of 103° C. She was delivered of twins, both of whom died of streptococcal meningitis within 24 hours of their birth. The first 2 swabs taken from the husband were negative for haemolytic streptococci but the 3rd swab, taken three days after the first, gave a profuse growth. This strain, the nose strain of the patient, and the one meningeal strain tested were all identical with the infecting strain. A strain isolated from the throat of the doctor who attended the patient after her admission to hospital was not of this type. Nose swabs of husband and doctor were missing. This patient is included among those " confined at home " because she had acquired her infection before she was admitted to the institution where the delivery took place.

In the case of these 5 patients there is clear evidence to show that the contact strain was responsible for the infection.

(5) *The patient* (*CHA* 7).—This patient was attended by 2 nurses and a waiting woman. From the throat of the latter and the nose of the patient, strains identical with the infecting strain were recovered. The streptococcus isolated from the gums of the patient was not of this type.

In this case there is nothing in the evidence to indicate which of the strains was the source of the infection.

Summary

For the infection of 14 of the 63 patients investigated a possible source was found in the respiratory tract of both patient and contact.

Although, when a patient is herself carrying an extra-genital strain of the same type as the infecting strain, the possibility that this strain was the immediate cause of the infection cannot be excluded without an elaborate procedure of swabbing (p. 16), in the case of 13 (20·6 per cent.) patients the evidence points clearly to a contact strain as the source, and in the case of 10 of these 13 patients a history of pre-existing nasopharyngeal infection in the contact, or of her association with another case of puerperal fever, greatly strengthens the evidence ; in the case of the remaining patient confined in her own home, there was nothing in the evidence to favour either possibility.

2. Patients for whose Infection a Possible Source was found in the Patient Only

A. *Six patients whose Attendant Contacts, so far as could be Ascertained, were all Swabbed*

(*a*) *A patient confined in an institution*

(1) *The patient P*2 (*OAK* 19), * *p.* 23.—This patient was delivered in a nursing home by a doctor who at the time of the confinement was suffering from quinsy, and who immediately afterwards went off duty. Eight days later another patient, P1, was delivered by another doctor. Strains identical with the infecting strains of the two patients were found in the nose of the patient P2, and in the throat of the doctor of the patient P1. Two of the six members of the nursing staff were carrying strains of haemolytic streptococci in their throats, but these strains were not of the same type as the infecting and other strains. There would seem to be no doubt that the infection was conveyed to the second patient by an undiscovered carrier or by utensils, etc. There is no evidence to show whether this patient acquired her nose strain directly from this source or indirectly from her uterine discharges.

It would seem to be very unlikely that the extra-genital strain of the patient was responsible for her infection.

(*b*) *Patients confined in their own homes*

(1) *The patients P1 and P2* (*ARM* 1).—These two patients, who were not in any way connected with each other, were delivered, within 3 days of each other, by the same midwife. The uterine and throat strains of the patients were identical but swabs from the nose and throat of the midwife yielded no haemolytic streptococci. To explain these facts it must be supposed either that, by chance, the two patients were carrying in their throats identical strains of a type prevalent at the time and that they both infected themselves from these strains, or that the midwife carried the infection in her instruments, utensils, etc., or that, for one reason or another (p. 33), the results of swabbings of her nose and throat were fallacious.

In the case of these patients the evidence is inconclusive.

(2) *The patient* (*LUF* 16).—The strains isolated from the nose of the patient was the only one identical with the infecting strain. At the autopsy, from the pericardium and from a thrombosed vein were isolated strains which were " green," that is, showed no beta-haemolysis on aerobic culture (p. 41)†. On the appearance of such cultures alone it might have been concluded that the patient was infected with two distinct organisms. Anaerobic culture, however, showed all the infecting strains to be indistinguishable, and subsequent tests revealed them to be of the same serological type.

(3) *The patient* (*PAG* 20).—The infecting strains of this patient were investigated on account of a relapse which occurred in her clinical condition, accompanied by a positive blood culture, 5 days after apparent recovery from a mild infection. The possibility that she had acquired a secondary infection was under consideration. The tests, however, showed that the uterine strain isolated on admission, and the blood strain, were identical with each other and with strains isolated from the patient's nose and throat on admission. From the latter site two strains not of the same serological type were recovered.

(4) *The patient* (*WIL* 49).—The only strain identical with the infecting strain was isolated from a focus on the foot of the patient which had been septic for some days before the confinement.

In the case of these 3 groups the epidemiological evidence indicates that the extra-genital strains isolated from the patient were responsible for the infection.

B. *Five patients with Attendant Contacts whose Noses and Throats were not all Swabbed*

(*a*) *A patient confined in an institution*

(1) *The patient P2* (*BRI* 4), * *p.* 23.—In a nursing home, two patients, who, so far as was ascertained, were in no way connected with each other, were delivered within 24 hours of each other by different doctors. Strains of the same serological type were found in the genital tracts of the patients, in the throats of the patient P2, and of the doctor of the patient P1, but no strains were found in the respiratory passages of the 4 members of the staff of the home who were examined. It is not possible to decide on the evidence whether the infection started from the throat strain of the doctor of the patient P1, or from that of the patient P2. Since it would appear to be extremely unlikely that 2 patients in a home would be infected independently at the same time by strains of the same serological type, transference of the organisms by an undiscovered carrier, or by utensils, etc., must be postulated, and it may well be that from this unsuspected source all the strains—both genital and extra-genital—were acquired.

In this case the evidence is inconclusive.

† See footnote to p. 41.

(*b*) *Patients confined in their own homes*

(1) *The patient* (*LAW* 14).—A strain identical with the infecting strain was found in the nose of the patient.

(2) *The patient* (*WHI* 33).—The strain isolated from the nose of the patient was identical with the infecting strain.

(3) *The patient* (*WOO* 34).—A strain identical with the infecting strain was found in the throat and gums of the patient. No final decision was reached as to the identity of the doctor's throat strain (p. 57), but the weight of evidence was definitely against its being of the same type as the infecting strain.

In the case of each of these 3 patients one contact nose swab only was missing. Since in this series haemolytic streptococci were recovered from only 4–5 per cent. of contact nose swabs and it is reasonable to assume that many of these strains were not of the human pathogenic type (p. 8), the fact that these swabs were missing does not materially reduce the probability that the extra-genital patient strains were responsible for the infections.

(4) *The patient* (*TIP* 29).—A strain identical with the infecting strain was found in the throat of the patient.

Since no swabs were examined from the contacts of this patient it is not possible to come to any decision as to the source of the infection.

Summary

An extra-genital strain identical with the infecting strain was found only in the patient in 11 of the 63 patients investigated. In 5 of the groups, however, contact material was incomplete.

A study of the epidemiological data shows it to be highly probable that 6 (9·5 per cent.) of the 63 patients did actually contract their infection from their extra-genital strains ; in the case of 4 patients the evidence is inconclusive ; in the case of 1 patient it points clearly to a contact strain as the source of the infection.

3. Patients for whose Infection a Possible Source was found in a Contact only

A. *Twelve Patients whose Nose and Throat Swabs yielded no Haemolytic Streptococci and 1 Patient who showed a Throat Strain not identical with the Infecting Strain*

(*a*) *Patients confined in institutions*

(1) *The patients P1 and P2* (*HAR* 12) * *p.* 19.—Three patients became infected within a month of each other. Their infecting strains were identical with each other and with the strains isolated from the throats of two nurses in the maternity department. The swabs of 5 other nurses gave no haemolytic streptococci.

(2) *The patient P1* (*BRI* 4) * *p.* 22.—A strain identical with the infecting strain was isolated from the throat of the doctor.

(3) *The patient P1* (*OAK* 19) * *p.* 21.—A strain identical with the infecting strain was isolated from the throat of the doctor, who was suffering from quinsy at the time of the confinement.

(4) *The patient P1* (*SMI* 24) * p. 25.—A strain identical with the infecting strain was isolated from the throat of a nurse.

(*b*) *Patients confined in their own homes*

(1) *The patient P*3 (*BUC* 4*a*) * *p.* 24.—A strain identical with the infecting strain was found in the throat of the midwife.

(2) *The patient* (*CROC* 8).—This patient was attended by a doctor, a nurse and a handywoman. The throat of the nurse and the handywoman yielded profuse growths of haemolytic streptococci, but the nurse's strain alone was identical with the infecting strain.

(3) *The patient* (*R.TA* 28).—The only possible source found for the infection was in the throat of the nurse.

(4) *The patient* (*CROS* 38).—Before the confinement the patient was nursing her landlady, who was suffering from "influenza," and who, in turn, attended on the patient at her confinement. The nurse arrived for the confinement when the head was on the perineum. The throats of the nurse and of the landlady gave profuse growths of strains identical with the infecting strains.

(5) *The patient* (*BOD* 3).—This patient was attended by the district staff of a hospital. Twelve contacts (6 members of her family and 6 doctors and nurses) were swabbed. The swabs of 3 of the 4 children and 1 nurse gave strains of haemolytic streptococci. One of the children suffered from a chronic ear discharge. The strain isolated from the pus, from the throat of this child and from the throats of two other children were all identical with the infecting strain, but the throat strain of the nurse was not of this type.

(6) *The patient* (*COO* 39).—The husband of this patient developed tonsillitis 1 day before his wife's confinement. From his nose and throat strains identical with the infecting strain were isolated and no other strain was found in the respiratory passages of the 4 persons in attendance on the patient.

(7) *The patient* (*M.ED* 41).—The only strain identical with the infecting strain was isolated from the throat of the patient's father, who had suffered from tonsillitis 1 month before the confinement.

(8) *The patient* (*MAS* 46).—For some days before the confinement this patient had been dressing her boy's septic finger. This finger yielded the only strain of haemolytic streptococci found to be identical with the infecting strain.

(9) *The patient* (*VEN* 31*a*).—This patient was delivered on the district of a general hospital. Swabs of 13 doctors and nurses were examined: 6 of them yielded haemolytic streptococci. The only one of these strains so far tested—the throat strain of the student who delivered the patient—was found to be identical with the infecting strain.

In the case of 13 of these 14 patients there is clear evidence to show that a contact strain was the source of the infection. The source of the infection of the patient P1 (BRI 4) is discussed on p. 22.

B. *Patients whose own Nose and/or Throat Swabs were Missing*

(*a*) *Patients confined in institutions*

(1) *The patient* (*BUS* 5).—In the maternity department of a general hospital two puerperal patients became infected at the same time, but no material was available from the first patient, who died. This woman became pyrexial on the 5th day of the puerperium. Two days later her child, who had undergone a clean operation in the surgical department of the hospital, and who had visited her mother the day after she was confined, developed tonsillitis. Two days later the 2nd puerperal patient (BUS) became febrile. *Cultures* of strains isolated from the uterus of this patient, the throats of the child and of 4 nurses in the maternity department were sent to this laboratory. The full number of the swabs examined is not known. The strains of the child and of 2 of the nurses were identical with the infecting strain. Each of 2 nurses was carrying in her throat strains of 2 serological types.

(2) *The patient P*2 (*SMI* 24) * *p.* 23.—Two patients within a week of each other developed peritonitis in the maternity department of a general hospital, in which sporadic cases of scarlet fever had occurred shortly before the confinements. On inquiry it transpired that a nurse from the wards in which cases of scarlet fever had occurred had been allowed to take duty also in the maternity department. Eight members of the staff of the maternity department were swabbed. From the throats of the first patient and three nurses, and from the nose of another nurse, strains of haemolytic streptococci were isolated, but only one of these strains—that of a nurse—was found to be identical with the infecting strains.

(3) *The patient P*4 (*CUR* 9) * *p.* 18.—*Cultures* of strains isolated from the throats of 5 nurses in the maternity department of a general hospital were received. The strains of 2 of these were identical with the infecting strain.

(*b*) *Patients confined in their own homes*

There was a history of recent nasopharyngeal infection in the carrier member of the household of the following three patients:—

(1) *The patient* (*AVE* 35).—A strain identical with the infecting strain was recovered from the throat of the patient's mother, who had a sore throat shortly before the confinement. No other swab was examined.

(2) *The patient* (*L.BRO* 37).—A strain identical with the infecting strain was isolated from the nose of this patient's child, who had "had a cold" a few days before the confinement. A strain recovered from the throat of the nurse was not of this type.

(3) *The patient* (*SMI.N.*48).—Strains of haemolytic streptococci were recovered from the throats of the patient's four children, who, it is stated, had had tonsillitis within a month of the delivery, and from the throat of the nurse in attendance. The nurse's strain and the two of the children's strains tested were found to be identical with the infecting strain.

In the case of these 6 patients consideration of the epidemiological data affords strong presumptive evidence of the importance of the contact strain in the causation of the infection, although the fact that the patient material was defective prohibits any speculation as to a possible part played by the patients themselves. In the case of the 3 patients confined in an institution, however, it would seem that even if extra-genital strains had been present in them the chances of their infecting themselves during the labour or in the puerperium would have been very slight.

(4) *The patient* (*JON* 45).—The daughter of this patient, who slept in the same room with the patient, developed scarlet fever on the 5th day after the confinement—the day on which the mother was admitted to hospital with puerperal fever. The actual date of the onset of the mother's pyrexia is not stated. The strain isolated from the throat of the child was identical with the infecting strain. Although the presumptive evidence that the infection passed from the child to the mother is strong, the possibility that the mother may have been carrying in her throat a strain which infected her own genital tract and the throat of her child cannot be excluded.

(5) *The patient* (*SMI.F.*47).—Strains identical with the infecting strains were isolated from the throats of the child of the patient and of the housekeeper (the handywoman).

(6) *The patient* (*D.TA.*27).—A strain identical with the infecting strain was found in the throat of the doctor.

The evidence in the case of these 3 patients is inconclusive.

Summary

A strain identical with the infecting strain was found in a contact only of 23 of the 63 patients investigated.

In the case of 19 (30·2 per cent.) of the 63 patients there is no reason to doubt that the contact strain was responsible for the infection : in the case of 6 of the 19, although their own noses and/or throats were not swabbed, the epidemiological evidence incriminating the contact strain is strong. In the case of the remaining 4 patients, whose nose and/or throat strains were missing, there is nothing in the history to point to a definite conclusion.

4. Patients for whose Infection a Source was not Found

A. *Four Patients who, themselves, and whose Attendant Contacts, so far as could be Ascertained, were all Swabbed*

(*a*) *Patients confined in an institution*

(1) *The patient* (*NEG* 50).—Strains were isolated from the throat of the patient and from the throat of the doctor, but they were not identical with the infecting strain. The swabs of her two attendants in the nursing home were negative for haemolytic streptococci.

(*b*) *Patients confined in their own homes*

(1) *The patient* (*BIR* 2).—The strains which were isolated from the throats of 2 nurses were not identical with the infecting strain.

(2) *The patient* (*CLA* 40).—A strain was isolated from the throat of the nurse but it was not of the same type as the infecting strain.

(3) *The patient* (*HAL* 11).—The strain isolated from the throat of the midwife was not identical with the infecting strain.

B. *Ten patients who themselves were not Swabbed, or whose Contacts did not yield the Full Complement of Swabs ; and a Patient whose Doctor's throat Strain was Lost*

(*a*) *Patients confined in institutions*

(1) *The patients P1 and P2* (*BEV* 36).—Three patients became infected in a hospital : 2 of the infecting strains were identical but the 3rd strain was not of their type. *Cultures* of strains isolated from the contacts were supplied. It was stated, however, that no strains were found in the swabs of the persons who had actually attended the deliveries. The strains isolated from the throats of the doctor and of 3 nurses were not of the same type as the two infecting strains. Owing to the unsatisfactory nature of the material, no further investigation was made with the 3rd infecting strain.

(2) *The patients P1 and P2* (*GUM* 42).—A patient confined in the maternity department and a patient delivered on the district of a hospital, within 4 days of each other, were infected with identical strains. The first patient, whose baby was born before the arrival of the nurse, was " attended to " by a neighbour, whose nose was not swabbed. The swabs of the nurse who delivered the second patient were missing ; a haemolytic streptococcus was isolated from the throat of a nurse who attended her in the puerperium, but this strain was not of the same type as the infecting strains.

(3) *The patient* (*HOD* 44).—Data relating to the delivery of this patient in a nursing home were not supplied, but it is believed that the full number of swabs was not received. The onset of the fever did not occur, however, until a week after her discharge from the home on the 10th day. Strains were isolated from the throats of two midwives in the home, but neither was of the same type as the infecting strain.

(4) *The patients P1 and P2* (*VAN* 31).—Two patients were infected in a maternity home. *Cultures* of the infecting strains and of a strain isolated from the throat of one nurse were sent to this laboratory. It was stated that all other throat and nose swabs were negative for haemolytic streptococci. The infecting strains of the 2 patients were of the same type but the throat strain was different from them. The throats and noses of the patients were not swabbed.

(*b*) *Patients confined in their own homes*

(1) *The patient* (*BIN* 1*a*).—Although the complete number of swabs was supplied, the strain isolated from the throat of one doctor had died out in culture before the strains of the group were investigated. The only other strain isolated—that from the throat of another doctor—was not identical with the infecting strain.

(2) *The patient* (*MAR* 17).—One of the children of this patient was suffering from otitis media : from the purulent discharge, and from the throats of another child and the handywoman, strains were recovered of which none proved to be of the same type as the infecting strain. No contact nose swabs were examined.

(3) *The patient* (*SAU* 23).—The strains isolated from the patient's throat and gums and from the throat of the nurse were not of the same type as the infecting strain. The contact nose swab was missing.

(4) *The patient* (*SOP* 25).—The strains isolated from the patient's throat and the nurse's throat were not of the same type as the infecting strain. The nurse's nose swab was missing.

Summary

In each of 3 of these 12 patient-contact groups 2 patients were infected with identical strains : this fact shows that in these cases an undiscovered extra-genital strain was responsible for the infections. In 8 of the 12 patient-contact groups (11 patients) the full number of swabs was not received.

The epidemiological conclusions relating to the above 63 patients are summarised on p. 30.

D. Conventions observed in Tabulating the Data

In this section and in Tables IV and V are summarised the results obtained in the experiments with the strains of the patient-contact groups already described.

Since it sometimes happened that, when there was more than one patient in a patient-contact group, the contacts of the two or more patients were not the same persons, the figures relate to individual patients. No importance was attached to the total number of contact strains recovered in each group, since it is obvious that this figure will vary with the conditions under which the delivery takes place. For example, the total number of throat strains identical with the infecting strain isolated from midwife contacts of a patient delivered in an institution may be as many as 4 or 5, whereas the figure for a patient attended in her own home by one midwife cannot exceed 1; or, again, the number of such strains isolated from members of the family of the patient when, say, a case of tonsillitis has occurred among them shortly before the confinement, is likely to

vary with the size of the family. The contacts of each patient, then, are grouped together, and for practical purposes treated as one individual.

Unfortunately, for rather more than half the patients the full complement of patient and contact swabs was not available. The effect of this deficiency on the results varies according to the particular objective in view. If, for example, a contact nose swab is missing, this fact will be of no importance if a strain identical with the infecting strain is found in any other contact nose swab, since no attention is paid to the total number of such strains recovered in any patient-contact group. If, on the other hand, no strain identical with the infecting strain is recovered from any other contact nose swab in the group, the (incomplete) material of this group contributes no evidence as to the frequency with which strains identical with the infecting strain are to be found in the contact nose, and its significance for the determination of a possible source for the infection will depend on whether or not a strain identical with the infecting strain is found in another situation, that is, in the patient's nose or throat or in a contact throat.

E. Definitions

(1) "*Complete material*"

This term is applied to :—

(*a*) Material comprising nose and throat swabs of the patient and, so far as could be ascertained, of all persons who actually took part in the conduct of the labour, including, in many instances, the "handywoman." Such material was available for the investigation of the source of the infection of 30 patients.

(*b*) Material which, in spite of one missing attendant-contact nose swab, was effectively complete, since another attendant-contact nose swab yielded a strain identical with the infecting strain (1 patient of 1 of the above groups).

Recognition of the fact that a strain identical with the infecting strain may be recovered from the respiratory passages of members of the household of the patient (p. 30) has led in certain cases to the swabbing of the noses and throats of these persons, and the experience gained in this study clearly shows that "complete material" should, strictly speaking, include these swabs. In this classification, however, such a wide interpretation has not been given to the term : in six only of the above-mentioned 30 patient-contact groups were such swabs taken.

(2) "*Incomplete material*"

This term is applied to material which was defective in either the patient nose or throat swab, or all attendant-contact nose or all attendant-contact throat swabs ; or in one or more nose or throat swab from a group of attendant-contacts of whom no other yielded either a nose or a throat strain identical with the infecting strain.

Table V. shows that the material derived from 32 patients and their attendant-contacts was incomplete in some way : 16 patients supplied nose and 21 patients throat swabs, while the group of attendant-contacts of 4 patients supplied nose and the group of attendant-contacts of 27 patients throat swabs.

Note.—In (1) and (2) above, 2 groups appear twice : in each of them there was a patient for whom material was complete and one for whom it was incomplete.

There was no conscious selection of patient-contact groups in which an extra-genital strain was found in the patient and not in a contact, or vice versa, or in both patient and contact, or in the nose and not in the throat, or vice versa.

The material was derived from 55 patient-contact groups : 41 patients represented sporadic infections and 22 patients small epidemics of puerperal fever (2 patients in each of 8 groups and 3 patients in each of 2 groups).

F. The Incidence of a Possible Source of Infection in the Respiratory Tract or Elsewhere in 67 Patients and their Contacts

A possible source was found for the infection of 48 patients. (Table V.)

1. *The respiratory tract.*—In the "complete material" group (31 patients) a possible source for the infection was found in the respiratory tract for 25 (80·7 per cent.) patients : in the patient 15 (48·4 per cent.) times, in the group of contacts 20 (64·5 per cent.) times and in both patient and contact 10 times. No source was found in the respiratory tract for 6 patients of 6 groups.

In the "incomplete material" group (32 patients) a possible source was found in the respiratory tract for 21 (65·6 per cent.) patients. No source was found in the respiratory tract for 11 patients of 8 groups.

Taking together all material derived from 63 patients and their contacts, a possible source of infection was found in a strain of the respiratory tract for 46 (73 per cent.) patients, while no source was found in the respiratory tract for 17 (27 per cent.) patients : the possible source of infection was found in 24 (38·1 per cent.) of the patients, in 36 (57·1 per cent.) of the groups of contacts and in both patient and contact 14 times.

Thus the figures show that a strain identical with the infecting strain was found about one and one-half times as often in the contact as in the patient. Naturally, if all the contacts are regarded as separate individuals the ratio, particularly in the case of patients becoming infected in institutions, is very much higher.

Swabs from the gums of 93 patients yielded 10 (11 per cent.) strains of haemolytic streptococci : in 5 cases the patient was also carrying the organism in her nose and/or throat. Six of these gums strains were examined serologically : in 2 cases both the throat and the gums strains were identical with the infecting strain : in

2 cases the respiratory tract strain, but not the gums strain, was identical with the infecting strain: in 1 case the infecting strain was not identical with the throat or the gums strain, whose relationship to each other was not determined.

2. *A septic focus outside the respiratory tract.*—Such a possible source of infection was found for 2 patients: in the one case in the septic foot of the patient and in the other case in the septic finger of a child of the patient, both foci having been infected before the confinement.

No source was found for the infection of 15 patients: in the case of 4 only of these patients was the full number of swabs received from patients and attendant-contacts: in one case only were the children of the patient swabbed.

In each of 3 of the groups in which material was incomplete 2 patients were infected with identical strains.

Owing to technical irregularities occurring in the experiments with them, the relationship of the other strains of the group to the infecting strain was not determined in the case of 4 patients of 4 groups.

A strain identical with the infecting strain was isolated from—

(1) the doctor	.. .. ..	in	5	patient-contact	groups.
(2) the midwife	.. .. ..	,,	15	,, ,,	,,
(3) the handywoman	.. ..	,,	3	,, ,,	,,
(4) the husband of the patient		,,	3	,, ,,	,,
(5) the mother or father of the patient	.. .. ..	,,	2	,, ,,	,,
(6) a child of the patient	..	,,	8	,, ,,	,,

In addition to the strains isolated from the noses and throats of patients and contacts a strain identical with the infecting strain was found in—

(1) the ear of a child of the patient,
(2) the septic foot of the patient,
(3) the septic finger of the child of the patient.

These 3 septic conditions were present before the confinement. The strains isolated from the 2 latter foci were the only possible source of infection found in the complete material of the 2 groups.

Summary of the Results

If these figures are further analysed in the light of the attempt described on pp. 18–27 to determine, by a study of the epidemiological evidence, which of the extra-genital strains was actually responsible for the infection in each of the 48 patient-contact groups which showed an extra-genital strain identical with the infecting strain, it will be concluded that 6 of the patients contracted their infection from their own extra-genital strain, and 33 from a contact strain, which in the case of 9 patients was isolated from a member of the household of the patient: in the case of the remaining 9 patients there is no point of evidence on which to decide which of the extra-genital strains was responsible for the infection.

G. The Distribution of Strains Identical with the Infecting Strain in the Nose and Throat Respectively of Patient and Contact

(Table V)

The patient.—In patients a nose strain identical with the infecting strain was found about as often as a throat strain.

The contact.—The grave defects in nose material prohibits exact comparison. While there appears to be a considerable preponderance of throat over nose strains, the figures for the " complete material " show that the possibility that the nose may play an important part in the causation of infection must never be overlooked.

While the effect on the apparent distribution of nose and throat strains among patients and contacts is commensurate with the degree of defect, the influence on the figure for the incidence of a possible source in one of these situations will obviously be less great. Moreover, the main defect was in the contact nose swabs. If, with due recognition of the fact that statistically the data were very scanty, the figures for the incidence of strains identical with the infecting strain in the " complete material " group be accepted as a rough index of the average distribution of the organisms in the nose and throat of the contacts of cases of puerperal fever of this type, it will be seen that the incidence of such strains in the throat is to the incidence in the nose as 3·8 is to 1. Any incompleteness in the nose swabs, then, will be relatively unimportant. Actually 5 only of the possible 32 contact throat swabs were missing as compared with 28 contact nose swabs.

H. The Significance for the Parturient Patient of Nasopharyngeal Infection in her Contacts

In 12 of the 13 cases investigated in this series contacts who had suffered from an inflammatory condition of the nose or throat or the accessory parts *shortly before the confinement* were found to be carrying strains identical with the infecting strains. This fact shows that the presence of persons in such condition in her environment entails a grave risk to the parturient woman who is not adequately safeguarded against infection.

Eight of these 12 persons were members of the household of the patient: 5 of them had suffered from tonsillitis, 1 from an acute cold, and 2 from ear discharge. In 6 of these groups the strains found in the member or members of the household were the only strains identical with the infecting strains but in 2 of these groups swabs of the patient and/or her attendants were not taken. In addition in 3 other families there was a carrier of a strain identical with the infecting strain who developed tonsillitis a few days after the confinement, but there was nothing to show whether the invasion of the throat had preceded or followed the confinement.

Further reference is made on p. 39 to the strains isolated from members of the household of the patient.

I. The Incidence of the Serological Types described by Griffith in a Series of Infecting Strains

Reference to Table VI and p. 53 will show that 85 of the 121 infecting strains tested gave a definite reaction on the slide with a serum, and that 7 types accounted for 66 per cent., and 11 types for 84 per cent. of all strains typed.* These are the types commonly met with in tonsillitis, otitis media, scarlet fever, etc. (Griffith, 1935). The type most frequently met with in this series of puerperal cases was the Type I Scarlet Fever type, infecting patients of 14 patient-contact groups; Type II Scarlet Fever type was third in frequency, infecting patients of 8 patient-contact groups; the other two Scarlet Fever types appeared less often.

J. Discussion of the Results

1. *Factors affecting the Correctness of the Figures*

A number of factors operate to give the figures for the incidence of strains identical with the infecting strain found in the nose and throat (Table V) a fictitiously low value.

(*a*) *Failure to secure the full complement of nose and throat swabs*

Tables IV and V show that in spite of the efforts made there was a lamentable lack of "complete material" in the groups investigated. In the early days in this laboratory the importance of the nose as a possible source of infection was not recognised, but even after this was realised it was frequently impossible to secure the prompt return of nose swabs. Recently, with a view to ascertaining whether they might yield a higher percentage of "positives", nasopharyngeal swabs have been examined: records of the results of these swabbings are not yet complete.

The material contributed from sources outside this hospital was frequently not collected until some days after the onset of the fever or even until the occurrence of a second case. Patient nose and throat swabs were commonly missing, and in some cases the patient died without their being secured. Occasionally it happened that one or other attendant had left the scene of the confinement before the swabbings took place. Towards the latter part of the inquiry a more efficient organisation resulted in the return of "complete material" in a higher proportion of cases, and the practice of investigating cases not admitted to this hospital had been discontinued.

Swabs were seldom taken from members of the household of the patient. The figures (p. 30) show the potential importance of these swabs, however, particularly when there is a history of any inflammatory condition of the throat or nose in any member of the family.

The possibility cannot be excluded that in certain cases in the absence of doctor or nurse, as for instance in the case of a "B.B.A." delivery, well-meant but unskilled and, for the patient, truly hazardous attentions were given to her by a neighbour who was not regarded as a "contact".

* *See* Note to Table VI.

(*b*) *Failure to isolate a haemolytic streptococcus from the swab*

The results of single swabbings which are negative for haemolytic streptococci must be accepted with reserve. On three occasions when circumstances pointed strongly to a particular source of infection, the first swab yielded no haemolytic streptococci, but a repeat swab gave a profuse growth of the organism. Moreover, on more than one occasion the swab taken from the nose or throat, which was subsequently proved to be carrying a strain of the same type as the infecting strain, yielded only a few colonies on the plate, and, in one instance, one colony only.

The importance of securing a really effective contact between the tonsil and the swab is not always appreciated. L. Colebrook (personal communication) in this laboratory failed to recover any haemolytic streptococci by direct culture from the mouth (saliva) of 13 out of 20 persons who gave a history of recent throat infections and whose tonsils at the time gave an abundant growth; 5 of the remaining 7 swabs yielded a scanty growth; 2 only gave an abundant growth. This finding, however, apparently conflicts with the results of the experiments of Paine (1935) described below (p. 34).

The fact that in this series the patients yield on the whole a higher percentage of nose strains than the contacts, may be, to some extent, attributable to the more favourable conditions under which the swabs of the former are taken, namely in hospital by a medical officer accustomed to the technique.

An infected antrum or nasal sinus may show an intermittent discharge of microbes; in one such case swabbed in this laboratory two negative swabs were followed a week later by a swab which yielded a profuse growth of haemolytic streptococci.

It is conceivable that a swab taken from a very dry nasal mucous membrane might fail to show a truly representative flora.

In a few instances two serological types have been isolated from a throat swab. It is clear that in such cases unless distinctive colonial appearance leads to the recognition of the two types the colony picked for the stock culture may not be a colony of the strain responsible for the infection of the patient.

A completely sterile plate strongly suggests either faulty technique in swabbing, or the use of a relatively potent solution of antiseptic gargle or paint immediately before the swabbing. Instances have occurred during the course of this study in which nurses have deliberately resorted to such measures in order to avoid the risk of being found to be carrying haemolytic streptococci in their throats. In one such instance a sterile plate resulted from the first swab, but a repeat swab taken without warning gave an abundant growth.

(*c*) *Possible failure of the technique used to reveal identity between strains in every case*

Reasons have been given for the confidence placed in the technique of the absorption experiments employed for the identification of strains. There is, however, admittedly a slight risk that, exceptionally,

the technique used might fail to reveal the evidence of identity between strains, which did, in fact, exist. The experiments were designed and the standard of identity in the interpretation of the results was adopted (p. 14) to safeguard against the possibility that serological relationship between strains might be mistaken for identity and that irregularities in the behaviour of sera or strains might lead to false conclusions of identity.

Attention has been called to the possibility that negative results in direct (slide) agglutination may be due to an insensitive suspension. In 5 groups (7 patients) in which negative results were obtained, in 2 of which (3 patients), so far as could be ascertained, material was complete, the slide tests alone were used for the identification of strains.

2. *The Mode of Spread of the Infection*

The study of the cases described in this report affords no solution of the problem of the mode of transference of these organisms from the respiratory passages of the patient or contact to the genital tract. There is at present no evidence to show whether the vagina or perineum is contaminated directly during the labour, by spraying, through coughing, speaking or sneezing, or indirectly through the medium of hands or instruments accidentally soiled by spraying or by contact with other contaminated objects; or whether the microbes are conveyed by the patient herself before, during or after the labour by hands contaminated by contact with the secretion of her own respiratory tract or that of members of her household by handkerchiefs, etc.; or through the medium of bedding, utensils, towels, etc. The possibility that the infection may be acquired during the early hours, or even days, of the puerperium must not be ignored. In a case reported by Paine (1931) infection appeared to have been acquired from an attendant who was not present during the labour, and in one case of this series such a happening could not be excluded, but it is possible that the infection was derived from this attendant by the contamination of dressings or utensils before the delivery.

In this connection the following experimental evidence is of interest. L. Colebrook (1933) found that while transference of these organisms by spraying from healthy carriers seldom occurred, it could be demonstrated readily when a recently inflamed throat was carrying an abundance of haemolytic streptococci. Paine (1935), on the other hand, studying the momentum of droplets, was able to demonstrate 10 positive cultures when 14 healthy carriers *coughed* on to blood-plates held a few inches from their mouths. He regards the discrepancy between the results of his and L. Colebrook's experiments as due to the different positions in which the plates were held for the experiment.

L. Colebrook and Maxted (1933) showed that the antiseptic technique commonly in use is quite inadequate to cleanse the hands of streptococci, or to prevent their early re-infection with organisms even if they have undergone an initial disinfection.

3. *Comparison of the Findings in the Present Research with those of Smith* (1931 *and* 1933) (see *Table V*)

In his two series relating to infections of similar type to those studied in this series and comprising 49 patients in 39 patient-contact groups, for all of whom, it is stated, material was complete, Smith found a possible source of infection in the respiratory tract for 39 patients of 29 groups, a source outside the respiratory tract for 2 patients of 2 groups, and no source for 8 patients of 8 groups. The findings are thus in general agreement with those of this study, the only striking difference being the less frequent occurrence in Smith's series of a strain in the nose of the patient identical with the infecting strain: this and the somewhat lower incidence in his series of such strains in the throat of the patient is associated with his relatively infrequent isolation of such strains in both the patient and her contact.

4. *The Type of the Infecting Strains*

The puerperal strains tested belong to a limited number of common serological types. This fact, if further experience should show that they are representative of the majority of puerperal infecting strains, has a potentially important bearing on any problem of immunisation or serum therapy which may arise. To what extent the incidence of these types corresponds with their seasonal incidence in other diseases due to haemolytic streptococci has not yet been shown, but since they are the types commonly met with in other haemolytic streptococcal diseases, it is reasonable to assume that prevalence of certain types in the general community will be reflected in the frequency of their appearance in puerperal cases.

In other words, so far as is known at present, there is nothing to suggest that the streptococci responsible for puerperal fever are different in type from those which cause the common diseases of the respiratory tract and other pathological conditions. This fact, in itself, lends strong indirect support to direct experimental evidence that the strains found in the respiratory tract are responsible for puerperal infections.

IV.—THE PRACTICAL APPLICATION OF THE RESULTS

In this section an attempt is made to correlate the available facts and to estimate the practical significance of the results described for the problems of maternal mortality and morbidity.

(1) *Are the haemolytic streptococci responsible for puerperal infection to be found in the genital tract at the beginning of labour: that is, is the infection, in the strictest sense, endogenous?*

The evidence at present available (p. 9) all goes to show that the haemolytic streptococci which give rise to puerperal fever are rarely, if ever, present in the genital tract at the beginning of labour. It must, therefore, be assumed that the organisms found in infected puerperal women were introduced from some extra-genital source during labour or in the early days of the puerperium.

(2) *Is there any fruitful source of the organisms which are capable of initiating infection to be found elsewhere in the patient or in the persons in her immediate environment ?*

It is known (p. 10) that in the human respiratory tract is found the most important source of the haemolytic streptococci which are pathogenic for human beings. Moreover, it has been shown in this study (Table VI, p. 32) that the uterus-infecting strains belong to the same serological types as the organisms isolated from patients and the contacts of patients suffering from tonsillitis, scarlet fever, etc. The experience gained supports the experimental evidence of Hare (1934) in associating organisms recovered from recently inflamed throats with a high degree of risk of infection if a parturient woman is exposed to them.

(3) *In what percentage of cases infected with haemolytic streptococci was an extra-genital strain of the organism actually found in patient or contact ?*

Dr. Elizabeth White, in this laboratory, investigated the source of the infection of a consecutive series of 197 puerperal patients admitted to the hospital in 1933 and 1934 suffering from haemolytic streptococcal infection of various degrees of severity. In the case of 122 of these patients, the patients themselves and their immediate attendant-contacts only were swabbed. In the case of 75 patients the members of their households were swabbed in addition : in many instances there was a history of recent acute or chronic infection of the respiratory passages in a member of these 75 families. An extra-genital strain of the organism was found in patient or contact in the case of 148, that is, 75 per cent. of these 197 patients : from one or more member—excluding the patient herself—of 41 (54 per cent.) of the 75 families examined a strain of haemolytic streptococcus was isolated.

Reasons are given in the text (p. 33) for the belief that nose and throat swabbing as at present practised fails to reveal the full number of carriers. Moreover, it is certain that the practice of restricting the swabbing to patients and to persons actually in attendance on them, or of including members of the family only when some point in the epidemiological history points to the likelihood of their being carriers, leads to results which are fallaciously low.

Although, however, it is not unlikely that a sufficiently comprehensive search for the source of the infection would reveal the organism in one or other extra-genital site in all, or nearly all, cases, the figures actually obtained, that is 75 per cent., must be taken as the basis for the present computations.

(4) *What percentage of extra-genital strains examined serologically were shown to be identical with the infecting strain ?*

Of 31 patients in this series and 49 patients in the two series examined by Smith (1931 and 1933), who, themselves, and, so far as could be ascertained, whose attendant-contacts were all swabbed,

an extra-genital strain serologically identical with the infecting strain was found in the case of 27 (87 per cent.) and 41 (83·7 per cent.) respectively (Table V). For the infection of these 80 patients, then, a possible source was found in patient and/or contact in 85 per cent. If to these are added the 32 patients and their contacts in this series from whom the full complement of swabs was not obtained, the percentage of cases for whose infection a source was found is still as high as 79·5 per cent.

It has been suggested (p. 32) that owing to certain technical fallacies inherent in the methods used and to the critical standard adopted in the interpretation of the results, the above figure (87 per cent.) for the cases studied in this work is almost certainly too low. The figure 85 (based on the 80 cases), however, represents the percentage of cases for whose infection a source has actually been demonstrated in the conditions in which the problem has been studied hitherto.

(5) *In what percentage of the cases admitted to hospital with haemolytic streptococcal infection can a source for the infection be demonstrated ?*

The serological findings referred to under (4) above apply to selected cases (p. 16), the majority of whom were suffering from infection of a severe type. In view of the facts shown under (1), (2) and (3), however, there appears to be no obvious reason why the figure 85 per cent. should not be applicable to the 197 patients under (3) above, and, at any rate, to all patients whose infection is sufficiently severe to necessitate their removal to hospital, and who, therefore, presumably, are infected with organisms of the human pathogenic group. The figures quoted under (3) and (4) above, then, show that in the case of 85 per cent. of 75 per cent., that is, of 64 per cent. of patients admitted to hospital the serological identity of the infecting strain with the extra-genital strain can be demonstrated ; this fact, since no other source for the infection can be discovered ((1) above), affords the strongest presumptive evidence that the infection was acquired from these extra-genital strains.

(6) *Is it possible to prevent the entry of these extra-genital strains to the genital tract ?*

Statistics published from time to time by the officers of maternity institutions shew an incidence of infection which is very much lower than the average. Three well-authenticated examples may be given :—

(i) At the East End Maternity Hospital it was arranged that during the 12 months ending May 31st, 1933, a swab should be taken for bacteriological examination of every woman having on at least one occasion after the first 24 hours of the puerperium a temperature of 100° F. or more for which no definite extra-genital cause could be assigned. These swabs were examined by Dr. L. Colebrook (personal communication). 1,892 women were delivered

during that period: 1,441 in hospital and 451 in their own homes. Only one woman, who was never seriously ill, proved to be infected with haemolytic streptococci. This was a patient who was delivered on the district: her child, who had slept in the mother's bed, had impetigo due to a haemolytic streptococcus.*

(When the case records for the 1,441 hospital deliveries were subsequently scrutinised, it was found that a swab had not been taken from 14 cases who had shown slight pyrexia; the fever, however, was so slight as to make it appear very improbable that any of the cases were infected with haemolytic streptococci of the human pathogenic group (p. 8).)

(ii) At the Elsie Inglis Memorial Maternity Hospital (1935) a bacteriological examination was made of all febrile cases occurring among 2,095 women delivered in the hospital during the years 1933 and 1934. From only 5 of these cases was a haemolytic streptococcus isolated, that is, once in every 400 deliveries.

(iii) At University College Hospital, London (Reports for 1931, 32, 33) for several years a bacteriological examination has been made of the cervical swab of every morbid puerperal case—with the exception of those cases whose morbidity was clearly due to an extra-genital cause. From 2,896 booked cases delivered in the Obstetric Unit during 1931–1933 haemolytic streptococci were isolated only three times in association with a "morbid" puerperium —giving an incidence of infection by this organism of approximately 0·1 per cent.

There is no reason to suppose that the carrier rate (of extra-genital strains) in these patients and their contacts differed in any way from that in such persons in other areas.

Such figures would seem to dispose of the possibility, for which, incidentally, there is no contributary evidence, that patients known to be carrying a strain identical with the infecting strain in their own respiratory passages have acquired their uterine infection via the blood stream. In this series in no case was there evidence to point to any recent inflammatory reaction of the patient's nose and/or throat from which the strains were isolated.

Clinical experience, then, lends support to the experimental evidence in showing that by the use of scrupulous care, an intelligently devised antiseptic technique and adequate masking the risk of infection may be successfully overcome. Discussions of the measures appropriate to secure this end have already appeared in the press (Interim and Final Reports of the Departmental Comm. of the Min. of Health on Maternal Mortality and Morbidity, 1930 and 1932; L. Colebrook and Maxted, 1933; Paine, 1935). Certain points may be emphasised here:—

(*a*) A bacteriological examination of a swab from the uterus of every febrile puerperal patient, and a search for carrier strains,

* Unfortunately, owing to technical difficulties with this uterine strain, no conclusion was reached as to whether the child's strain was identical with the infecting strain.

when the diagnosis of haemolytic streptococcal infection is established, is highly desirable. The finding of haemolytic streptococci in the uterus will point to the potential seriousness of the infection, and the discovery of an extra-genital strain will lead to the adoption of adequate measures for the protection of other patients. (*See* also L. Colebrook and Griffith, 1930).)

(*b*) It is not generally feasible at the present time to conduct a prophylactic bacteriological survey of patients and their attendants to determine the incidence of organisms of the human pathogenic group (p. 8). But, when possible, such an inquiry is of great practical value in directing attention to the need for the strictest vigilance in the presence of a carrier of such organisms, to the particular danger which appears to be associated with strains inhabiting recently inflamed respiratory passages (p. 31), and to the importance of removing a patient from a heavily infected environment.

(*c*) The findings of this study clearly indicate the potential danger which arises from those extra-genital strains which may be designated as "familial," that is, strains derived from the patient and the members of her household, as distinct from those strains which may be designated for convenience as "professional" and are introduced by her attendants. It has been shown above (p. 36) that in 54 per cent. of the families examined a relation of the patient yielded a strain of haemolytic streptococcus and (p. 30) that in the case of 15 of the 48 patients for whose infection a possible source was found, a familial strain appeared to have initiated the infection. Now it must be emphasised that these figures do not refer to a random sample of swabs taken from the noses and throats of the members of the households of the patients. While on the one hand the relations of a patient are more likely to be swabbed when a history of tonsillitis, for example, is elicited, and while the patient-contact groups which included strains isolated from such familial carriers were to some extent selected for serological investigation on account of their intrinsic epidemiological importance, on the other hand it is obviously highly probable that in the case of other patients, owing to the failure to seek for it, the possible source of infection in the family was overlooked.

While, then, it is admitted that the real risk to which patients are exposed from these familial strains cannot be estimated from this material, the figures appear fully to justify the contention that wherever in her environment streptococci of the human pathogenic group are lurking they are potentially dangerous for the parturient patient and to show that the successful tracing of the source of infection involves a far more comprehensive search than has hitherto been made.

Since it must be presumed that in certain cases the uterine infection is acquired either directly from the contaminated hands of the patient, or, perhaps, indirectly from her bedding, utensils, towels, etc., there is clearly an urgent need for the education and assistance of the patient in the practice of scrupulous personal cleanliness,

of the disinfection of her hands, and, even on occasion, of the use of a mask.

It is believed that when the exact nature of the problem of infection by these organisms is fully realised, an effective technique to prevent their introduction to the genital tract will be devised and employed.

(7) *To what extent would the practice of measures which successfully prevented the introduction of haemolytic streptococci to the genital tract reduce the present maternal mortality and morbidity rates?*

The following deductions are based on the fact that in 64 per cent. of cases ((5) above) an extra-genital source for the infection can be demonstrated:—

(*a*) *Mortality.*—Approximately 3,000 maternal deaths occur in each year in England and Wales as a direct consequence of childbirth. The Departmental Committee of the Ministry of Health (1930, 1932) estimated that about 40 per cent., that is 1,200, of these maternal deaths are due to sepsis. Haemolytic streptococcal infections account for 75 per cent., that is 900, of the deaths from sepsis. At least 64 per cent. each year in England and Wales, that is at least 576 of these 900 deaths are preventible.

(*b*) *Morbidity.*—The mortality rate for cases infected with haemolytic streptococci under the best conditions of medical and nursing care is accepted as at least 20 per cent. (p. 7). A death rate of 576 per annum, therefore, represents a sickness rate of approximately 2,880 per annum. By the prevention of these 2,880 infections in each year in England and Wales the 2,304 women who ultimately recover would be saved from sickness—a large number of them from a prolonged and distressing period of invalidism and from subsequent sterility or anxiety to avoid the risk of further pregnancy.

These computations are based on the fact that with the current bacteriological procedure a source of infection has actually been demonstrated in 64 per cent. of cases. Reasons have been given, however, for the supposition that a more comprehensive search, more effective swabbing and a perfected serological technique will lead to the detection of an extra-genital source for all or nearly all haemolytic streptococcal infections. If and when that ambition is realised, it will be justifiable to argue that by the use of a reliable obstetric technique all or nearly all haemolytic streptococcal puerperal infections are preventible.

V.—THE BACTERIOLOGICAL TECHNIQUE

A. General Description

1. *The Isolation of the Organisms**

A swab is taken from the vagina or cervix of a patient suffering from puerperal pyrexia immediately after her admission.

* The isolation of organisms from the patients admitted to this hospital and from the contacts of these patients was carried out in the routine department of this laboratory by Dr. Elizabeth White.

This swab is smeared* on to a horse-blood agar plate† which is incubated anaerobically; after 18 hours' incubation, or on the following day if replating is necessary, if the plate shows haemolytic colonies, one of these is planted on to another blood plate. The surface of this plate which is to be incubated aerobically is streaked and at the same time a deep scratch is made in the medium with the charged loop. If careful search reveals haemolytic organisms of different colonial type on the first plate, a representative of each type of colony is picked.

The anaerobic incubation of the first plate is practised in order to detect the presence of those strains which do not give beta-haemolysis (Brown, 1919) when grown aerobically (Fry, 1933). In the case of the second plate, an organism which is non-haemolytic on the surface of the plate will show haemolysis along the line of the scratch where conditions in the depths of the medium are sufficiently anaerobic.‡

If there is no haemolysis along the line of the scratch the culture is rejected: if there is haemolysis, the organism is filmed, and if it proves to be a streptococcus, the soluble haemolysin test (McLeod, 1912) is performed with it. The test is repeated if a negative result is associated with a poorly growing culture. Only those strains which form a filtrable haemolysin are included in this study. A Robertson's meat tube is inoculated from a sweep of the colonies on the second plate.

Permanent stock cultures were originally kept in this meat medium, but when it was found that certain important strains had died, the practice of storing in meat was replaced by desiccation *in vacuo*: a few drops of a 20 per cent. serum broth culture, derived from the original meat culture within a month of isolation of the strain, are dried in an ampoule.

If the appearance of the organisms on the first plate appears to justify a provisional diagnosis of a haemolytic streptococcal infection, application is made forthwith for nose and throat swabs of the patient and her contacts; if the diagnosis is doubtful, this is postponed until after the performance of the soluble haemolysin test.

2. *The Serological Investigations* §

(*a*) *Preparation of the suspensions*

Every stock culture and suspension is plated before use.

Suspensions for the slide tests are first prepared.

* From this point the procedure is the same when a uterine swab or a culture is received from a patient not admitted to this hospital.

† *See* Appendix (c).

‡ Dr. Griffith (personal communication) has recently shown that these strains form β-haemolytic colonies when cultivated aerobically on the surface of blood plates prepared with nutrient agar of somewhat different composition from that used in this laboratory. An inquiry into the essential difference between the two media is in progress.

§ Full details of the preparation of the reagents and of the tests used will be found in Section B (p. 43).

A vaccine is made and a serum prepared from the infecting strain and, subsequently, from the strain to be tested by reciprocal absorption experiments. The (water-bath) agglutinating suspensions of the strains used in immunisation and the absorbing suspensions of these and other strains of the patient-contact group are prepared either at the same time as the vaccines, or later, shortly before the first sampling of the sera.

(*b*) *The tests*

(i) The infecting strain of a patient-contact group is first tested by *direct agglutination* on the slide with all *the absorbed sera*. If it is found to belong to one of the types represented, the other strains of the group are tested to determine whether they also belong to this type.

(ii) The *primary* (one sided) *absorption experiment* is performed with a serum prepared from this infecting strain : its titre in the water-bath and the minimum absorbing dose (*vide* p. 13) having been found, samples are treated with that number of cocci of the homologous strain, of some or all of the nose and throat and other strains of the patient-contact group, and of certain " control " strains whose selection is guided by the results of the preliminary slide typing. The treated samples are titrated with a suspension of the homologous strain in the water-bath.

(iii) The *reciprocal absorption experiment* is performed with a second serum prepared from one of the respiratory tract or other strains of the patient-contact group which had effected complete absorption of the first serum. This second serum in due course is treated with the homologous strain, with the infecting strain and with some or all of the other strains of the patient-contact group and with " control " strains.

Thus, for the most part, in the patient-contact groups selected for study by absorption tests, identity of the infecting strain and one respiratory tract strain of the group only was established by reciprocal absorption experiments. The relationship of the remaining strains of the group to the infecting strain was inferred from their behaviour as absorbing strains with the sera of the group, that is, from the results of one sided absorption experiments as well as from direct agglutination tests. With the strains of a few groups the reciprocal experiment has not yet been performed. In two groups a second serum was prepared from a throat strain which had not effected absorption of the first serum.

The absorption experiments with most of the sera were repeated once or more than once, in some cases after an interval of months or years.

(iv) *Direct agglutination tests with the unabsorbed sera* prepared from the strains isolated from the members of the patient-contact groups were set up in the water-bath with the suspensions of a number of strains.

(v) In addition a larger series of *unselected infecting strains* were tested with the Griffith absorbed sera by direct (slide) agglutination in order to ascertain the number of types occurring among puerperal strains.

B. Direct (Slide) Agglutination

1. *The Sera*

The sera used in the technique introduced by F. Griffith (1935) are prepared by a prolonged course of inoculations : they are repeatedly absorbed with massive doses of heterologous strains of known type, selected after extensive testing. The almost complete type-specificity of the resulting samples is demonstrated by a further series of tests with a large number of strains.

Twenty-seven of these sera as well as cultures of the homologous well-defined type strains were most generously contributed by Dr. F. Griffith. To one or other of these types belong the strains isolated by Griffith over a period of years from various human infections, chiefly from infections of the nose and throat. Repeated tests of these sera in this study have failed with very few exceptions to reveal any group agglutinins in them : the exceptions will be described in the section of " results."

2. *Preparation of the Agglutinating Suspensions*

A thick suspension is prepared from strains of the patient-contact group by growing them in 5 c.c. of trypsin phosphate broth (Appendix (b)) and collecting the settled deposit. Vigorous shaking on the mechanical shaker (Wright and Colebrook, 1921) immediately before use temporarily improves the character of the suspension. Certain strains tend to give an auto-agglutinated (" granular ") suspension, but if a number of cultures are put up, either in series or in parallel, it is usual to find among them one which is usable. The change from a granular to a smooth suspension has appeared in this study to occur in quite a haphazard manner, an improvement in one culture being followed by deterioration in the next, etc. Strains which are persistently granular in trypsin broth may give good suspensions in glucose phosphate broth (Appendix (a)) or in 5 per cent. ascitic trypsin phosphate broth. Owing to the tendency of the latter medium to decrease the agglutinability of the strains it has been used only when the other media have failed to produce a suitable suspension.

The method of using pooled sera, whereby the process of testing is considerably shortened, is described by Griffith (1935).

3. *The Test*

The type of the infecting strain of the patient-contact group is first sought. A stable suspension, that is, one which shows neither auto-agglutination nor saline sensitivity, having been obtained, it is tested with all the absorbed sera in succession, drops of suspension and sera being mixed on the slide. If no immediate reaction occurs, the slide is gently rocked. If by a strong reaction with one serum the type of the infecting strain is found, stable suspensions of some

or all of the other strains of the patient-contact group are tested with this serum, and, when a positive result is obtained, with a number of other sera acting as controls. A delayed or negative result calls for a repetition of the test with a new suspension. A persistently negative result must be taken to mean that the type of the strain is not represented among the sera.

The results are read with a low-powered binocular microscope.

C. The Absorption of Agglutinins

1. *The Significance of the Terms "Complete Absorption" and "Partial Absorption" as used in this Report*

Throughout the greater part of this work the routine practice has been to arrange the experiments so that the dilution of the serum in the absorbing mixture and in the first tube of the titration of the two-fold dilutions of the treated samples are 1/32nd and 1/16th of the titre respectively, with the end-point of agglutination in the 5th tube ("a 5-tube titration"). In such an experiment, samples of treated serum which show a reduction of titre by 2, 3, 4 and 5 tubes have suffered a 75, 87·5, 93·75 and 96·8 per cent. exhaustion of agglutinins. The significance of a one tube (50 per cent.) reduction is discussed below (p. 50). Thus, if the titre of a serum is 1/1,600, its dilution in the absorbing mixture is 1/50, and in the first tube of the titration 1/100 (1/32nd and 1/16th of the titre respectively). The agglutinin content of a treated sample of this serum which gives agglutination to titre (1/1,600) has suffered no reduction. The agglutinin content of a treated sample whose titre is 1/400, that is, in the 3rd tube, is 1/4th of its original value: the absorbing cocci have removed 75 per cent. of the agglutinins. Similarly the agglutinin content of a treated sample which gives no agglutination in any of the five tubes and whose titre is therefore not more than 1/50, is not more than 1/32nd (50/1,600) of its original value: the absorbing cocci have removed at least 31/32nds (96·8 per cent.) of the agglutinins.

2. *Preparation of Agglutinating Sera from Strains of the Patient-Contact Groups*

The great majority of the infecting strains used in immunisation of rabbits were taken from the original stock cultures within six months of their isolation—a number within a month or two. Since the selection of the strains used in preparation of the sera for the reciprocal experiments—mostly respiratory tract strains—was determined by the results of the primary absorption experiments, these cultures were somewhat older when the vaccine was prepared from them.

(*a*) *The routine procedure*

250 c.c. of 0·2 per cent. glucose phosphate broth (Appendix (a)) inoculated with 2 c.c. of a 6-8 hour culture of cocci in 20 per cent.

serum broth are incubated for 18 hours and subsequently heated for 30 min. at 55° C. The centrifuged deposit is suspended in 0·5 per cent. phenol so as to give a concentration of approximately 5,000 million cocci per c.c. Rabbits are inoculated intravenously twice or thrice weekly for 4 or 5 weeks, the first dose being approximately 500 million and the last dose 5,000 million cocci.

The serum is sampled at the end of 4–5 weeks: if the titre is below 1/1,600 or the quality of the reaction is poor, in order to determine whether the new agglutinating suspension or the serum is at fault, they are tested, respectively, against a good serum of the same type and a sensitive suspension of its homologous strain, if these are available. According to the results of these titrations, or in any case if such a serum and suspension are not available, the rabbit is given a further short course of inoculation after a rest of 6–8 weeks, and/or a new agglutinating suspension is prepared. If, on sampling after the second course of dosage, the reaction is not satisfactory a new vaccine is inoculated into another rabbit. It is the exception, however, for the serum at the first sampling to be unsatisfactory.

The sera are preserved with 0·5 per cent. phenol.

(*b*) *Departures from the routine procedure*

From six strains heat-killed vaccines were prepared as described but without the addition of preservative. They were stored in the ice-chest and tested for sterility from time to time during use.

After a preliminary test of heavier doses, which in most cases proved fatal, 6 rabbits were immunised with weekly doses of 2–10 million living cocci grown in the glucose phosphate medium. Four of these rabbits, in addition, received doses of dead cultures.

In certain cases the age of the primary and secondary cultures used in preparation of the vaccine and the routine of dosage were varied.

(*c*) *The quality of the sera prepared*

Eighty-four sera were prepared from 80 strains. Eight of these sera, which showed titres below 1/800, were not used in the absorption experiments. That the sera and not the agglutinating suspensions were defective was shown in the case of three of these sera by the fact that the three sera failed to react with sensitive suspensions of strains identical with the three homologous strains, while suspensions prepared from the three homologous strains reacted to titre with other sera prepared from strains identical with them. As the type of the strains from which the remaining sera were prepared was not known it was not possible to perform similar tests with them.

The titres of the remaining 76 sera and the relation of the titres to the character of the results of absorption experiments are shown in Table III and discussed on p. 54. With 67 sera satisfactory experiments which showed no more than insignificant variations from the standard set up, were performed. Certain of the experiments

with 10 sera were characterised by major irregularities : with five of these the evidence appeared to incriminate the sera, while in the case of the remaining five, three of which in addition gave satisfactory experiments and, are therefore, included in the 67 above, it seemed more reasonable to ascribe the discordant results to some abnormality of the absorbing suspensions, or even possibly to some undetected technical error. In addition, experiments with two sera of poor quality showed anomalous behaviour, probably of minor import, which was not fully investigated.

The quality of the sera prepared from heat-killed unpreserved vaccines did not appear to differ from that of those prepared from preserved vaccines.

No material advantage has at present appeared to accrue from immunisation with living cocci, while the disadvantages associated with the preparation of weekly inocula and the delay due to the rabbits' reactions have been considerable.

Variations in the age of the stock cultures and of the primary and secondary cultures used in the preparation of the vaccines and in the routine of dosage have not resulted in a demonstrable variation in the quality of the sera obtained.

3. *Preparation of Agglutinating Suspensions for use in the Water-Bath*

Various devices have been recommended for overcoming the marked tendency of haemolytic streptococci to give auto-agglutinating suspensions in broth. It would appear that some undetected variation in the quality of the broth considerably influences the character of the suspension, for certain batches of broth have been associated with better and others with worse results. There was no apparent correlation between the pH and the character of the suspension.

The repeated subcultures practised by Smith (1931, 1933) and others have been avoided, partly on account of the possibility that the antigenic structure of the strains might be modified thereby, and, partly, because the method did not seem to yield consistently promising results, the character of the culture being either unchanged or changed in quite a haphazard way, an improvement in one sub-culture being followed by a relapse in the next.

Shibley (1924) reported that cultures grew uniformly at room temperature (17°–23° C.) but that granularity developed with even a short period of incubation at 37° C. While his claim for the value of slow growth at these temperatures has been substantiated in general, no deleterious effect has been observed to follow relatively short periods of growth at 37° C.

After a short preliminary trial of other methods, the technique of slow growth in broth was adopted as the most promising. Fifty or 75 c.c. of 0·2 per cent. glucose phosphate broth (Appendix (a)) are planted with a few drops of an 18-hour-incubated 20 per cent. serum broth culture, a fresh culture or one that had been stored

for a few weeks in the ice-chest being used. Slow growth is obtained by leaving the culture at 20° to 23° C. or by subjecting it to alternate periods of warming at 37° C. and cooling on the bench or in the ice-chest, the first incubation being continued until there is just visible growth. In hot summer weather, even over-night growth on the bench may be too long, as the relatively rapid growth may give rise to clumping of the cocci throughout the medium. When it is judged that turbidity is sufficiently dense (an opacity of about 200 to 400 million cocci per c.c.) the culture is plated, formolised (0·25 per cent.), sealed with a waxed rubber cap and stored at room temperature.

With a reasonably docile strain the process may be completed in 2 days : with a refractory strain the rate of growth must be more retarded and the required turbidity may not be obtained in less than 4 days.

From careful examination of the culture with a hand lens in a good light half an hour after vigorous shaking, one can predict with confidence whether or not the suspension will be usable. A good suspension will always remain homogeneous for two hours, usually for several hours and not infrequently for two or three days : a finely-granular deposit will ultimately settle out, but the cocci will not be aggregated and they will readily go up into uniform suspension and remain up again after vigorous shaking. Very rarely will such a suspension agglutinate with saline in the water-bath ; in other words, generally speaking, if there is no spontaneous aggregation there is no salt sensitivity. In the water-bath such suspensions give perfectly reliable readings for four or more hours : even when the tubes are left overnight in the ice-chest and the cocci have settled in the control tube in a fine powdery deposit, the distinction between this and the agglutinated clumps is perfectly clear: if the deposits are dispersed by gentle shaking, the suspension in the control tube will remain uniform after shaking has been discontinued, whereas in the tubes in which agglutination has occurred the clumps will slowly re-form. If such a suspension is too thin it is concentrated by settling.

In another type of suspension which will not remain homogenous either on the bench or in the water-bath for two hours there is partial early aggregation of the heavier particles. If these are allowed a few hours in which to settle the still turbid supernatant fluid will be found to be usable.

The suspensions are shaken vigorously immediately before use. Although success has not necessarily attended the first effort, and although suspensions have varied in agglutinability, no strain has been met with in the last two years from which a suspension could not be prepared. The present stock consists of suspensions of 96 strains—a fair sample of all which have been prepared : many of these have been re-tested at intervals of months or years : they have retained their stability and, with few exceptions—apart from those whose original quality was indifferent—they have shown little

or no loss of agglutinability. The majority of them are perfect suspensions as judged by any standard; some of them show a fine granularity which, however, in no way prohibits the reading of the end-point of agglutination with a hand lens.

On a very small scale comparison was made between formolised and unformolised agglutinating suspensions: 16 suspensions were prepared from 8 strains of different type: one suspension of each strain was stored without formalin in the ice-chest, the remainder were formolised in the usual way. Two titrations were performed with each suspension at intervals of some weeks. The stability of the unformolised suspensions remained unimpaired and their titres did not differ appreciably from those of the preserved suspensions.

4. *Preparation of the Absorbing Suspensions*

The cocci are grown at 37° C. in 100–250 c.c. of 0·2 per cent. glucose phosphate broth inoculated with 1 to 2 c.c. of an 18-hour-old 20 per cent. serum broth culture. In order to stimulate growth the flask is gently shaken during the early part of the incubation at intervals of about half an hour. At the end of 18 to 24 hours the culture is plated and its opacity estimated by Brown's tubes. The deposit obtained by centrifuging is re-suspended in 0·25 per cent. formol saline at a concentration of 20,000 million cocci per c.c. and stored at room temperature. No difference in absorbing power was detected between formolised and unformolised suspensions of 12 strains, or between old and freshly formolised suspensions of 4 strains.

5. *The Tests*

(*a*) *Determination of the M.A.D.*

If there is a liberal supply of the absorbing suspension, samples of diluted serum are treated simultaneously with the various absorbing doses; otherwise the first dose tested is 20,000 million cocci per c.c. of diluted serum and, according to the result of this test, other samples are treated with 2,500, 5,000, 10,000 or 40,000 million cocci until that dose is found at which absorption is complete.

The exact procedure will be made clear by an example of an experiment with a serum whose titre is 1/1,600, in the titration of which the end-point is to be in the 5th tube (a "5 tube titration"), and whose first absorbing dose is to be at the rate of 20,000 million cocci per c.c. (8,000 million for 0·4 c.c.). 8,000 million cocci are suspended in 0·2 c.c. of saline. To this is added an equal volume of the serum diluted to 1/25 (1/64th of the titre). The dilution of the serum in the "absorbing mixture" is now 1/50 (1/32nd of the titre). A mixture of equal volumes of the 1/25 dilution of the serum and saline constitutes the "untreated sample" or serum control. The mixtures are gently shaken at frequent intervals at room temperature. At the end of half an hour the absorbing mixture is centrifuged and the serum is pipetted off. One volume (0·2 c.c.) of the homologous agglutinating suspension is added to one volume (0·2 c.c.) of the treated sample and of the untreated sample. The mixtures, together with a saline control of the suspension, are incubated in the water-bath for 2 hours at 55° C.

The tests with other absorbing doses are set up in exactly the same manner.

If the 40,000 million dose is insufficient to remove the agglutinins from the serum, since it is difficult to deal accurately with a volume of cocci larger than 40,000 million per c.c., one of the following alternatives must be chosen:—

(1) The serum may be diluted to 1/37·5 or 1/50; its dilution in the absorbing mixture will then be 1/24th or 1/16th of the titre respectively; in the former case there may still be very slight agglutination in the 5th tube, but in the latter case a titration with the end-point in the 4th tube will be set up.

(2) Successive doses of 30,000 million or even of 40,000 million cocci per c.c. may be used; in this case 0·4 c.c. of serum diluted to 1/50 (1/32nd of the titre) is added to the centrifuged deposit of cocci; after 10 minutes the mixture is centrifuged and the serum transferred to the second deposit.

In the earlier part of this study the former, and in the later part the latter, expedient was preferred.

The M.A.D. is taken as the smallest number of cocci which effects complete absorption, or a number not more than twice as great as that which leaves a trace of agglutinin in the treated sample. It was rarely as small as 5,000 million, frequently as large as 40,000 million, four times 80,000 million and once 120,000 million.

(*b*) *The primary and reciprocal absorption experiments*

The sera for these tests having been selected as explained on p. 42, they are treated in this way, at the M.A.D., with the absorbing suspensions of strains of the patient-contact set and with control strains (p. 14). In this case, however, the degree of absorption is to be determined by the titration of the treated sample. To allow for this titration, in which 0·4 c.c. of each sample is required, the volume of the absorbing mixture must not be less than 0·6 c.c. In each experiment a sample of serum treated with the homologous strain is included.

In the titrations, 0·2 c.c. volumes of six two-fold dilutions of the untreated sample and of each treated sample are mixed with 0·2 c.c. of the homologous agglutinating suspension, and these, together with a saline control of the suspension, are incubated in the water-bath at 55° C. for two or four hours.

(*c*) *The reading of the results*

The results are read against an illuminated dark background with a hand lens.

A full description of the results and a discussion of their significance will be found on p. 53, *et seq*. They are considered here only in so far as they involve an extension of the experiments or a modification of the technique.

In the majority of the experiments at the end of two hours in the water-bath the untreated sample has agglutinated to titre ; " complete absorption " by the homologous strain and by other strains, if any, known to be identical with it, is contrasted with " no absorption " by strains known to be of different serological type from the homologous strain. " Unknown " strains have absorbed either completely or not at all. In the reciprocal experiments there has been complete absorption of each serum by the strain from which the other serum was prepared.

In certain experiments a 50 per cent. (one tube) drop in the titre of either the untreated sample or the samples treated with heterologous strains is seen. Its significance in the case of the untreated sample lies in the fact that the occurrence automatically changes the experiment from a 5-tube to a 4-tube titration experiment, thus giving the term " complete absorption " a 93·75 per cent. instead of a 96·8 per cent. value (p. 44). In the case of the treated samples, following the line taken by G. S. Wilson (1925) this occurrence has been considered to be " as likely . . . due to faulty technique as to definite absorption and . . . . therefore of no great moment ".

In a number of the experiments certain variations from the two patterns described above have been seen.

Minor degrees of partial absorption (75 per cent. or less) at the M.A.D., which have occurred not infrequently, call for a repetition of the experiment with a higher absorbing dose. This is readily achieved when the M.A.D. is a low one, but when it is already high at a 1/32nd-of-the-titre dilution a two-fold higher dilution (1/16th of the titre) must be used, with the same or a larger absorbing dose. In this case it is not strictly accurate to express the absorbing dose of the second test in terms of the M.A.D. of the first test, since the M.A.D. at a two-fold higher dilution is likely to be somewhat less than half that at the lower dilution. For practical purposes with these organisms, however, it has been found that, in order to avoid a re-test of the M.A.D. in each case, it may be taken to be between 1/2nd and 1/3rd of the first figure. Complete absorption by an " unknown strain " at the same absorbing dose with the new dilution may be accepted as evidence of serological identity between the strains and the homologous strain ; incomplete absorption at a 3 × M.A.D. dose as evidence of serological dissimilarity. Demonstration of the inability of a strain to absorb completely at an even higher dose will ratify the conclusion.

A definite partial reduction in the amount of agglutination in each tube of the samples treated with heterologous strains may complicate the reading of the results, more especially when, as occasionally happens, the untreated sample shows poor agglutination

in the last three or four tubes. Usually this difficulty can be overcome by the postponement of the reading of the results to four or even six hours, always provided that at that time the sample treated by the homologous strain shows no trace of agglutination. If this expedient does not provide the solution of the problem, it must be sought in the use of another agglutinating suspension, or in the repetition of the experiment with a lower serum dilution (1/64th of the titre) since the additional tube in the titration at the lower dilution shows a larger volume of flocculation, and a correspondingly larger residue after treatment, than the tubes at the progressively higher dilutions.

A major degree of absorption (>75 per cent.) by one or more heterologous strains prohibits the drawing of conclusions from the experiment. Since such a high degree of absorption by one strain believed to be of different serological type from the strain from which the serum under absorption was prepared may be due to technical error or to some peculiarity of the absorbing suspension, a repetition of the experiments with the same and with another suspension of the strain is indicated. If the high degree of absorption by this strain, A, of the serum prepared from the strain B, persists, doubt is thrown upon the original assumption that the two strains, A and B, are of different serological type : to decide the point a reciprocal absorption experiment must be performed, that is, a serum prepared from the strain A must be treated with the strain B. Failing evidence to the contrary (*see* serum P Ut 44 (WOO 34) p. 56), the occurrence of such a high degree of absorption by two or more heterologous strains of different serological types reflects on the quality of the serum : the remedy is the preparation of a new serum.

In the earlier stages of this work a drop in titre by two or more tubes in the untreated sample was occasionally seen. This appeared to be associated with the practice of storing samples of diluted serum, either before or after their treatment with the absorbing suspensions, in the ice-chest for one or two days before the titrations were performed. Since the experiments were completely invalidated by the occurrence they do not appear in the detailed description of the results. After the practice of storing the diluted samples of sera was stopped and the experiments, once started, were completed without delay, no more than 50 per cent. drops in titre occurred and these were regarded as being within the limits of the experimental error.

In certain cases a repetition of an experiment has revealed some inconsistency in the behaviour of different absorbing suspensions of a strain (p. 59).

D. Direct (Water-Bath) Agglutination with Unabsorbed Sera

The sera and the agglutinating suspensions were those used in the absorption experiments.

Reciprocal agglutination tests were set up, in batches of 8 to 16 strains and sera at a time, one volume (0·2 c.c.) of each serum,

diluted to 1/32nd of the titre, being put up with an equal volume of a suspension of each strain. These mixtures, together with saline controls of the suspensions, were incubated in the water-bath at 55° C. for 2 or 4 hours.

In addition, whenever agglutinating suspensions of the strains used for absorption in an experiment were available, they were tested against one dilution (1/32nd of the titre) of the untreated sample of the serum at the time of the performance of the absorption experiments.

VI.—THE BACTERIOLOGICAL RESULTS

The technique which has been described has developed as the result of the experience gained throughout this study. A general survey of the technical possibilities and difficulties of the methods, rather than prolonged exploration of any particular aspect of the problem, was aimed at. For these reasons the experiments and the results submitted to some extent lack that desirable homogeneity which is obtainable with a well-tested technique whose limitations are clearly foreseen.

A. Direct (Slide) Agglutination with Absorbed Sera

This technique was used to identify the strains of certain selected patient-contact groups, and to determine the incidence of the types described by Griffith in a series of infecting strains.

1. *Identification of the Extra-Genital Strains of the Patient-Contact Groups*

Absorbed sera of 27 different types (p. 43) were used in these tests. It is believed that the strains from which 3 of these sera were prepared are types very infrequently met with in human infections: no representative of their type was found among puerperal strains. A very close serological relationship exists between the sera FG 15 and FG 17: in this study certain strains reacted with the first and one set of strains with the second serum, but no distinction between all these strains could be made out by absorption experiments. Two or three sera appeared to be incompletely exhausted of their non-type-specific agglutinins, and to give results which were not trustworthy.

Tests were performed with the infecting, the nose and throat and other strains of 52 patients and their contacts: the infecting strain of 51 of these patients gave a definite reaction on the slide: the infecting strain of 1 patient showed indefinite reactions with one serum.

The serological relationship between the infecting strain of 32 of these 52 patients and the extra-genital strains was determined by direct (slide) agglutination and absorption experiments: of 19 patients by direct (slide) agglutination only. In the case of 42 of these 52 patients one or more extra-genital strains identical with the infecting strain was found in patient and/or contact: in the case of 9 patients no such strain was found: in the case of 1 patient the results were inconclusive.

2. *Determination of the Type of the Infecting Strain in a Series of Unselected Cases of Definite Infection*

The type of the infecting strain of 136 patients was investigated. Four of the strains did not give usable suspensions. Twenty-five strains did not react with any serum: since, however, in no case were more than 3 suspensions of these strains tested it is possible that some at least of the negative results with them are attributable to failure to secure a sensitive suspension.

The infecting strains of 97 patients reacted strongly with an absorbed serum, but absorption experiments failed to confirm the type of one of these strains (p. 62).

Six strains showed an indefinite reaction with a serum and 4 strains reacted with 2 sera.

The results are as set forth in Table VI. It will be seen that the infecting strains of 85 patients (derived from 85 separate puerperal outbreaks) which reacted definitely with a serum were distributed among 18 Griffith types, and that 84·7 per cent. of all strains typed belonged to 11, 65·9 per cent. to 7 and 54·1 per cent. to 5 Griffith types.

With one exception (p. 20), in every case in which a survey of the epidemiological data suggested a common infection for two or more patients the infecting strains have been found by these tests to be identical.

Comment on these results is postponed until after the description of the measure of agreement found between them and the results of absorption experiments (p. 62).

B. Absorption of Agglutinins

1. *General Results*

The strains of 38 patient-contact groups (48 patients) were investigated by this method (Table V). 139 absorption experiments were performed with 76 sera. 595 samples of these sera were treated with absorbing suspensions representing 179 strains identical with the homologous strains, 256 heterologous strains, and 18 strains whose relationship to the homologous strain was not determined (Table II).

It has been explained (p. 42) that only a proportion of strains in each group were identified by reciprocal absorption tests. 52 sera were used in reciprocal absorption tests for the identification of 64 strains; in every case the results of straightforward one-sided and reciprocal tests were in complete agreement. The relationship to the infecting strain of all strains of the groups which were not tested by reciprocal absorption was determined by one-sided experiments.

From certain experiments with 5 sera in respect of all strains tested, and with 1 serum in respect of one strain, no conclusions were drawn.

In these experiments, in which all sera were absorbed at a 1/16th or a 1/32nd-of-their-titre dilutions, there was no apparent relationship between the size of the M.A.D. on the one hand and the titre of the serum, or the occurrence of anomalous results in the absorption experiments, on the other hand.

2. *Detailed Results**

The protocols of the experiments are given in full in Table I; they are summarised in Table II and on p. 61. No useful purpose would be served by a description of the individual technically satisfactory experiments. On the other hand, since the acceptance of the conclusions drawn from the results as a whole depends on the recognition of the character and frequency of occurrence of results which do not conform to this standard, these results are described in detail in order that critics who wish to do so may form their own judgment on their significance and cause.

(*a*) *Experiments showing either no irregularities or only insignificant ones*

In 110 experiments with 67 sera, 476 samples of sera were treated with absorbing suspensions of 165 strains identical with the homologous strains and of 218 heterologous strains.

(i) In 96 experiments with 61 sera, 134 strains identical with the homologous strains absorbed the serum completely. 103 strains at the M.A.D., 9 strains at a dose not greater than the 3×M.A.D. dose and 22 strains at an unmeasured absorbing dose.

(ii) In 12 experiments with 6 sera 17 strains identical with the homologous strain failed to absorb completely at the M.A.D. and the higher dose was not tested. Fifteen of these strains effected a degree of absorption only 3 per cent. less than the homologous strain. Two of these strains absorbed about 10 per cent. less than the homologous strain. One of these figures was obtained in an experiment in which four other strains identical with the homologous strain absorbed completely at the M.A.D.; the other referred to a Griffith type strain of which the absorbing suspension had shown itself to be relatively inactive in other experiments.

Absorbing suspensions of 203 heterologous strains effected no absorption in these experiments.

In 7 experiments with 7 sera, absorbing suspensions of 15 heterologous strains effected a 75 per cent. reduction of titre. In a repetition of the experiments one strain effected no absorption. Two of the sera were treated with an unmeasured absorbing dose greater than the M.A.D. The M.A.D. with three sera was so high as to preclude

* The designations of the strains and sera used in this section are those described in Table I (q.v.).

a test of an effectively higher dose. The M.A.D. of the remaining serum was a moderate one, but a higher dose was not used.

In addition 2 experiments with 2 sera (N_1 T 55b (CUR 9) and P Ut 42 (SPR 26) showed certain irregularities of this character. As neither serum gave a satisfactory agglutination reaction further tests were not made with them: in one case another serum was available, but a second serum prepared from the other strain showed no improvement on the first. These sera appear in Tables II and III as " unclassified ".

(*b*) *Experiments showing significant irregularities presumably attributable either to defects of the sera or to idiosyncrasies of the absorbing suspensions or strains.*

In 27 experiments with 10 sera, 3 of which also appear in experiments under (*a*) above, 110 samples of sera were treated with 11 strains identical with the homologous strains, with 35 heterologous strains, and with 15 strains whose relationship to the homologous strain was not determined:—

(i) *Experiments showing no absorption by one suspension of a strain identical with the homologous strain at the M.A.D. or at a dose greater than the M.A.D.*—The absorbing suspension 6/7/33 of the strain P_5 PF (CUR 9) was used in the experiments with three sera (N_1 T 55a; N_2 T 75a and P_1 Ut 76) prepared from strains identical with it of the patient-contact group (CUR 9). At the M.A.D. this suspension effected no absorption of the first two named sera, but complete absorption of the third serum; at a dose three times as great as the M.A.D. it failed to absorb at all the serum N_1 T 55a. The suspension 31/10/33 of this strain, however, absorbed completely the serum N_1 T 55a, the serum P_1 Ut 76 and the serum N_2 T 75b obtained by another bleeding of the rabbit 75; it was not tested with the serum N_2 T 75a. The serum P PF (EAS 10) was not absorbed by the strain of the group P Ut, but was completely absorbed by heterologous strains at the M.A.D. Further reference to this serum is made on p. 56.

(ii) *Experiments showing failure of a strain to absorb the homologous serum.*—The heaviest dose tested (40,000 million per c.c.) failed to effect any reduction of the titre of the serum P Ut 97 (WIN 33a); as it was completely absorbed at this dose by strains of two heterologous types, no further absorption experiments were made with it and the respiratory tract strains of the group were not tested with it.

(iii) *Experiments showing major degrees (>75 per cent.) of absorption by heterologous strains.*—The serum P_2 T1 74 (BRI 4), whose titre at the end of a 15 weeks' period of intermittent immunisation was 1/1,600, showed a marked reduction of titre (93·75 per cent. at the end of two hours in the water-bath, 75–87·5 per cent. at the end of 4 hours) in the samples treated with 7 suspensions of 6 heterologous strains. In this case collateral absorption experiments and direct agglutination confirmed the tentative differentiation of

these strains from those which effected complete absorption. The serum P Ut 105 (SEA 23a), whose titre at the end of two 4 weeks' periods of immunisation was 1/1,600, showed 75–93·75 per cent. absorption by two unknown and one heterologous strain. Investigation of this serum and group has not yet been completed. Another serum is being prepared.

Four sera showed complete absorption by heterologous strains at the M.A.D. :—

The serum P_5 PF 56 (CUR 9), whose titre at the end of 3 weeks' immunisation was 1/1,600, showed 93·75 per cent. absorption by two strains in one experiment but no absorption was effected by these same strains in a repetition of the experiments with this serum or in experiments with another serum prepared from the strain P_5 PF. There is unfortunately no record as to whether the absorbing suspensions were the same.

The titre of the serum P Ut 41 (BYR 6) at the end of $5\frac{1}{2}$ weeks' immunisation was 1/800, and as another serum prepared from this strain showed a titre of only 1/400 no further experiments were made with the strains of the group.

The serum P PF 59 (EAS 10) whose titre at the end of 4 weeks' immunisation was 1/800, was prepared from an old laboratory strain, which was included in this investigation because while the uterine strain showed normal beta-haemolysis under aerobic conditions, the peritoneal strain showed this type of haemolysis only in anaerobic culture. As there was collateral evidence of the identity of the two strains and as the source of the infection of this patient was not investigated a new serum was not prepared.

The serum P Ut 97 (WIN 33a), whose titre at the end of 20 weeks' intermittent immunisation was 1/400, was completely absorbed by two of the three heterologous strains of different type tested, at a dose at which the homologous strain effected no absorption (*see* (*b*) (ii) p. 55). The respiratory tract strains of this group were not tested. The behaviour of the serum in direct (water-bath) agglutination tests is described on p. 60.

The results of the experiments performed with the serum P Ut 44 (WOO 34) and with the serum P B 32 (NAS 18) (p. 57) appear to merit special attention. A detailed description of the results of the absorption experiments is given below accompanied by a note on the results of direct (slide and water-bath) agglutination tests, since these afford important contributory evidence.

Strains of the group (*WOO* 34).—The infecting strain, P Ut, the patient's throat strain, P T, the doctor's throat strain, D T.

Sera.—P Ut 44 : P T 85.

Type of the infecting strain.—F G 2.

This group was investigated on four occasions between January, 1933, and September, 1934. Unfortunately, the strain D T had died out in meat culture before the first repetition of the experiment.

A. *Slide typing.*—The serum F G 2 gave strong reactions with the strains P Ut and P T, but none with the strain D T.

B. *Absorption experiments.*—The full data are shown in Table I : they may be summarised as follows :—

1. *Primary absorption experiments. Serum P Ut* 44.—(α) At the M.A.D. the strains D T and P B (NAS 18), a strain used as a heterologous control, reduced the titre by 93·75 per cent., but even at a dose 20 times greater they failed to remove the last traces (3 per cent.) of agglutinin. The strain D T was not available for further tests. (β) In subsequent experiments at varying absorbing doses the strain P B (NAS) twice effected an 87·5 per cent. reduction and three times, in a different absorbing suspension, effected no reduction of titre. Details of the other absorbing suspensions are not known, but it is very probable that one suspension was used for the various experiments under (α) above.
2. *Reciprocal absorption experiments.* (α) *Serum P T* 85 (*WOO*).—At the M.A.D. this serum was completely absorbed by the infecting strain P Ut and was unaffected by the strain P B (NAS). (β) *Serum P B* 32 (*NAS* 18).—In 6 experiments the strain P Ut (WOO) did not reduce the titre of this serum.

C. *Direct agglutination in the water-bath.*—(α) The serum P Ut 44 (WOO) reacted strongly with the strain P T (WOO) and with other representatives of the Griffith type strain F G 2, but not with D T (WOO), P B (NAS) or with 36 other strains of various types. (β) The serum P T 85 (WOO) reacted strongly with the strain P Ut (WOO) and with two other strains of the type F G 2, but not with P B (NAS), nor with three other heterologous strains. (γ) The serum P B (NAS) gave no reaction with the strain P Ut (WOO).

Comments on the strains of this group.—The evidence then leaves no doubt that the strains P Ut (WOO) and P T (WOO) are identical and that they are serologically different from the strain P B (NAS). It is much to be regretted that owing to the premature demise of the strain D T (WOO), investigations with it were not completed. The balance of evidence seems to be definitely against its being of the same type as the infecting strain, however. The fact that the material reduction of titre occurred in samples treated on more than one occasion with two heterologous strains would seem to discount the possibility of attributing it to technical errors, as might be suggested by the inconsistent results obtained with different suspensions of P B (NAS). The titre of this serum was 1/51,200–1/102,400 ; in the direct agglutination tests in the water-bath, it gave no reaction with any heterologous strain. The period of immunisation of the rabbit was 5 weeks.

(iv) *Experiments showing a degree of absorption proportional to the size of the absorbing dose used, by certain "unknown strains," but no absorption at any dose by heterologous strains.*—The serum P B 32 (NAS 18) affords the only example of this type of result met with in this study.

Experiments performed in January and June, 1933.—In this patient-contact group there were the following strains :—A strain isolated from the blood (P B), the nose (P N), and the throat (P T) of the patient, and from the throats of a doctor (D T), two nurses (N_1 T) and (N_2 T), and a handywoman (Hw T).

Sera.—P B 32 ; D T 50 ; N_1 T 49.

Type of infecting strain.—F G 16.

On blood agar the strain P T showed very small colonies such as are not uncommonly isolated from normal throats but which have never in this study been found to be of the same type as an infecting strain. The cultures of the strain D T and N_1 T showed two types of colony, the one being rougher and more coherent than the other. Liquid cultures derived from single colonies of either type showed a mixture of the two types with a preponderance

of the type from which the culture was derived. No serological distinction between the colonies has been made out on the slide or in the absorption experiments.

A. *Slide typing.*—The infecting strain P B, and the strains D T and N_1 T gave variable reactions with the type serum F G 16. Other strains of the group either gave negative results with this serum or did not provide suitable suspensions for the test.

B. *Absorption experiments.* 1. *Primary absorption experiments. Serum D T* 50.—The strains of the group P N, D T, N_1 T and Hw T and the type strain F G 16, some of which on two occasions at the M.A.D. effected no absorption, at 16 times the M.A.D. partially, and at 50–100 times the M.A.D. completely absorbed the serum, while the other strains of the group and heterologous control strains, even at the heaviest dose, consistently failed to reduce the titre.

2. *Reciprocal absorption experiments.* (α) *Serum P B* 32.—At the M.A.D. the strains tested, namely, P B, N_1 T and F G 16, failed to absorb the agglutinins while the strain P N twice effected complete absorption. (β) *Serum N_1 T.*—At the M.A.D. the strains tested, namely, P B, D T and P N, failed to absorb at all.

Experiments performed in October, 1934.—When the original experiments were performed no special importance was attached to them since it was anticipated, in the light of published reports, that similar results would be not infrequent. When, with further experience of the technique used in this study, this outlook was found to be unjustified, the experiments were repeated. Unfortunately, at that time two only of the strains of the group, P B and D T, with their corresponding sera, remained for test.

A. *Slide typing.*—Both strains gave a moderate reaction with the serum F G 16.

B. *Absorption experiments.* 1. *Primary absorption experiments. Serum P B* 32.—At the M.A.D. the strain D T effected no absorption. At a dose about 13 times the M.A.D. the strains D T and F G 16 achieved partial absorption (75 per cent.). Complete absorption was not obtained, but it must be noted that in these experiments with agglutinating and absorbing suspensions which were different from those used in the previous experiment, the M.A.D. was about 10 times as large as before so that the heaviest absorbing dose was not so high a multiple of the M.A.D. as in the previous experiments.

2. *Reciprocal absorption experiments. Serum D T* 50.—No absorption was effected at the M.A.D. by the strain P B. As the M.A.D. of this serum was very high (60,000 million) no test of a higher dose was made.

C. *Direct agglutination in the water-bath.*—Each of the sera P B 32 and D T 50 agglutinated strongly the suspension of the homologous strain but not the suspension of the other strain.

Comments on this patient-contact group.—If the results of the absorption experiments conducted at the M.A.D. are to be judged by the standard adopted in this study there is no evidence that any of the respiratory tract strains of the group are identical with the infecting strain. The evidence of direct agglutination in the water-bath supports this view. On the other hand, direct agglutination on the slide suggests that, if not a type, at least an intimate non-type-specific antigenic group relationship exists between the infecting strain and just those nose and throat strains which at a massive dose effected complete absorption of the serum prepared from this strain, which other strains of the group and the heterologous control strains consistently failed to do. Moreover, it is noteworthy that one of these strains (P N) did actually completely absorb at the M.A.D. the serum D T 50. It is reasonable to suppose that if any two strains are identical, those others which behaved in a similar manner in the absorption of the serum P B 32 would share their type. The second set of experiments has done nothing to solve the riddle.

The titre of the serum P B 32 (NAS 18) was 1/51,000–1/102,400; in direct agglutination tests in the water-bath it did not react with any heterologous strain.

(v) *Experiments showing inconsistent behaviour of different absorbing suspensions prepared from a strain.*—Reference to Table I will show that the same absorbing strain was frequently used in the repeated absorption of a serum but there is in most cases, unfortunately, no record to show how often the same suspension of the strain was employed. It was not until two examples of a marked discrepancy between the degree of absorption effected by different suspensions of a strain occurred that the importance of this point was recognised.

The inconsistent results obtained in the experiments with the serum (P Ut 44 (WOO 34) p. 56) and with certain sera of the group (CUR 9) (p. 55) have been described.

With two other sera in otherwise straightforward experiments an absorbing strain showed the phenomenon in lesser degree. The fact that none of these strains has exhibited irregular behaviour in repeated tests with other sera suggests that there is nothing inherently peculiar in the strains themselves. Nor has direct agglutination in the water-bath revealed that they are distinguished from other strains by a preponderance of " group " antigens.

In no case has there been detected any falling off in the power of a strain (whether preserved in stock culture or in absorbing suspension) to absorb the homologous serum or the sera prepared from strains identical with it, although a marked change in the size of the M.A.D. has occasionally resulted from a change of agglutinating suspension.

3. *Discussion of the Results of the Absorption Experiments*

1. An unknown strain which effects a 75 per cent. reduction of titre of a serum in an otherwise straightforward experiment at the M.A.D. may be serologically identical with or different from the homologous strain: the use of a larger absorbing dose will decide the question: a strain identical with the homologous strain will absorb completely at a dose not greater than the 3 × M.A.D. dose: a heterologous strain will fail to effect complete absorption at this or a still heavier dose.

2. With the exception of the strains mentioned under (b) (i) in no case has a strain identical with the homologous strain failed to remove at least 90 per cent. of the amount of agglutinins removed by the homologous strain at that dose, and in every case tested (a) (i) and (ii)) a 3 × M.A.D. dose has proved adequate to complete absorption when the M.A.D. was insufficient.

3. A degree of absorption by heterologous strains which does not exceed 75 per cent. at this dose has not given rise to any difficulty in the identification of strains.

4. For these reasons the 110 experiments with 67 sera included under the heading (*a*) (p. 54) above are regarded as satisfactory

and conclusions as to identity of strains are drawn from them with confidence. The two specifically mentioned sera with which the tests were not concluded are excluded from these figures.

5. With one exception (*b*) (ii) (p. 55) every serum has been successfully absorbed by the homologous strain.

6. Although major degrees of absorption of sera by heterologous strains at the M.A.D. do not appear as a serious complication in this study, yet they have occurred with sufficient frequency to vindicate completely the principle laid down (p. 14) that heterologous control strains must be included in every experiment and that without such strains in no case can a verdict on the identity of "unknown" strains be accepted from experiments in which all the "unknown" strains tested effected complete absorption of the serum.

The experiments with 7 sera showed high degrees of absorption by heterologous strains. Consideration of the quality and titres of these sera is instructive, although it is recognised that from such small numbers only the most tentative conclusions can be drawn :—

In repetitions of the experiments with two of these sera the strains which had effected a high degree of absorption failed to absorb : the one (P Ut 44 (WOO 34) p. 56) was a serum of good quality whose titre was 1/51,200 : the other (P_5 PF 56 (CUR 9) p. 56) was a serum whose titre at the end of three weeks' immunisation was 1/1,600.

The remaining 5 sera (*b*) (iii), (p. 55) gave reactions which were so unsatisfactory that no further experiments were performed with them. While a careful review of the experimental conditions under which they were prepared and the absorption experiments performed reveals no departure from the usual technique nor any peculiarity in the strains or the cultures used in the preparation of vaccines, and agglutinating or absorbing suspensions, it does show that in each case the sera were exceptional in that the samples tested after the first 3–5 weeks' period of immunisation (8–12 doses) showed a titre of 1/800 or less. If a higher titre was reached with these sera by a prolonged course of inoculation this was not associated with any improvement in the quality of the sera as judged by the character of the results of the absorption experiments. Table III shows the apparent correlation between the titre value of the sera and the behaviour under absorption, and suggests that for this work sera of good titre—that is not less than 1/1,600—produced by a ready response in the animal body are indispensable.

The fact that in direct agglutination tests in the water-bath two of these recently prepared sera (P Ut 105 (SEA 23a) and P Ut 97 (WIN 33a)) showed an entirely unusual degree of reaction with a number of strains of heterologous type is additional evidence of the faulty character of the sera. Repeated tests (Chart I) with the serum P Ut 44 (WOO 34) on the other hand showed reactions only with strains identical with the homologous strain at the dilutions used. Unfortunately, direct agglutination tests were not performed with the other sera of poor quality, all of which were met with in the earlier part of this study.

7. The experience with the serum (P B 32 (NAS 18) (*b*) (iv) p. 57) is unique in this study. It has been explained that the technique of absorption was planned to avoid the potential risk that, by the use of very heavy absorbing doses heterologous strains might effect some high degree of absorption and so lead to an erroneous diagnosis of identity between strains. There has been little opportunity, however, to ascertain to what extent this risk is a real one with these organisms, since the M.A.D. of many of the sera investigated was so large at a 1/32nd-of-the-titre dilution that no more than a two-, three-, or, at most, a four-fold, increase of absorbing dose was tested. The serum P B 32 (NAS 18), however, was quite exceptional in having in the first tests an M.A.D. as low as 1,000 to 2,500 million per c.c., so that it was possible to set up tests in which the absorbing strains operated at a dose 50 or more times as great as the M.A.D. If the behaviour of the strains of the group which absorbed completely at that dose is to be regarded as an example of over-absorption by heterologous strains, however, it must be remembered that the heterologous control strains failed to absorb even at that dose. With this possible exception no such effect has been demonstrated in this series.

8. Provided that technical errors can be excluded with certainty it would seem probable that inconsistent behaviour of absorbing suspensions ((*b*) (v), p. 59) is associated with some physical property of the medium, or of the cocci, or with some change in the antigenic character of the strain. There has been no direct evidence to suggest this latter explanation, but in future work greater attention will be paid to the point and to the possibility of detecting such changes by distinctive colonial appearances. With a few strains, absorbing suspensions have been prepared from colonies showing individual variations, but in no case has a difference in absorbing power been detected by the relatively crude tests employed.

4. *Summary of the Conclusions drawn from the Results of the Absorption Experiments*

139 experiments were performed with 76 sera (Table II).

(1) 110 experiments with 67 sera were satisfactory or showed minor irregularities only : the results agreed with those obtained by the use of other techniques and deductions as to the identity of strains of the patient-contact groups concerned were made with confidence.

The investigation of 2 additional sera which showed irregularity of minor degree was not completed.

(2) 9 experiments with 3 of these 67 sera gave unaccountable results: from these and from an experiment with one other serum tentative conclusions only, or definite conclusions with regard to the identity of certain strains only, could be drawn.

(3) 6 sera gave completely unsatisfactory results from which no conclusions could be drawn.

C. The Extent of Agreement found between the Results of Absorption and Direct (Slide) Agglutination Experiments

1. *The Identification of the Respiratory Tract and other Strains of the Patient-Contact Groups*

The strains of 33 patients and their contacts were investigated by both techniques. In every case positive evidence of relationship between strains in the slide tests was confirmed by the absorption experiments, but in one case (group CUR 9, p. 63) the reaction on the slide failed to reveal the full number of identical strains of the group. In the case of 30 patients and their contacts, strains identical with the infecting strain were found in the respiratory tract or elsewhere: in 2 groups no such strain was found by either technique: the strains of 1 group gave doubtful results with both techniques.

2. *Determination of the Type of the Infecting Strain* *

Collateral absorption and direct (slide) agglutination experiments were performed for the identification of 32 strains.

27 strains reacted definitely with a serum on the slide: the validity of the results of the slide tests was shown in the case of 26 of these strains by the demonstration of complete absorption—(*a*) of the serum prepared from the infecting strain, or from another strain of the group, by the Griffith type strain homologous with the serum with which the strain under test had reacted on the slide (19 strains); and/or by an infecting strain of another group which the slide test had shown to be identical with the infecting strain under test (7 of the above 19 strains and 2 other strains); and (*b*) of the Griffith type serum in question by the infecting strain under test (5 strains). The two techniques failed to agree in the case of the strain P Ut (HAR 12) (p. 63): the serum prepared from this strain was not absorbed by the Griffith type strain nor was the strain P Ut able to absorb a serum prepared from a strain of another group, shown to be identical with this Griffith type strain.

Two strains which showed an indefinite reaction with a serum on the slide failed to absorb the Griffith type serum.

Three strains each reacted with two sera on the slide:—(*a*) The serum prepared from the infecting strain was not absorbed by either of the Griffith type strains nor was the one Griffith type serum tested

* The "Griffith type sera" used in the absorption experiments referred to below were the crude sera from which the absorbed sera used in the slide tests were prepared by Dr. Griffith.

absorbed by the infecting strain (group (BIN 1a)); (*b*) the serum prepared from the infecting strain was not absorbed by the one Griffith type strain; the other Griffith type strain has not yet been tested (group (CUR 9)); (*c*) the one of the Griffith type sera so far tested with which the infecting strain had reacted was completely absorbed by the infecting strain.

Details of the experiments with 3 of the strains referred to above are as follows:—

(i) *Group* (*HAR* 12)

On the slide the strains P Ut and N_1 T shown by reciprocal absorption to be identical reacted well with the serum F G 11, and three other infecting strains believed to belong to that type failed to effect any absorption of the serum P Ut 25. The identity of the two strains of the group, but apparently not their type, was indicated by the slide test. An absorption experiment with the serum F G 11 has not yet been performed.

(ii) *Group* (*CUR* 9)

In this patient-contact group were three sub-groups, A, B and C, represented by strains isolated on three occasions, separated by intervals of a few weeks' from three different patients, P_1, P_4 and P_5, and their contacts, in an institution. The infecting strain P_1 and certain strains isolated from contacts of the patient P_1 reacted with the serum F G 10, but this serum failed to identify these strains with certain strains of the sub-groups B and C with which subsequent absorption experiments showed them to be identical. The serum F G 10 was not absorbed by these strains of the sub-group A with which it had reacted on the slide, and the strain F G 10 failed to absorb the sera prepared from these and other strains of the sub-groups B and C identical with them.

In a batch of sera prepared from new types received later from Dr. Griffith one—F G 12—was found with which all the identical strains reacted. This serum was completely absorbed by the infecting strains of the three patients.

It appears then that the identical strains of the sub-group A were linked by a non-type-specific antigen as well as by the type-specific antigen and that this non-type-specific antigen was absent from or present in amount not detectable by the slide method in some or all of the strains of the other sub-groups which shared the type-specific antigen.

(iii) *Group* (*BIN* 1*a*)

The strains P Ut and P B reacted strongly with the serum F G 4 and to a variable amount with the serum F G 19—a serum which in this study has given not very reliable reactions. The serum P B 99 was not absorbed at the M.A.D. by the strain F G 4, by 3 other infecting strains shown to belong to this type, or by the strain F G 19. The serum F G 4, which was absorbed at the M.A.D. by the three infecting strains mentioned, was not absorbed at a dose 24 times as great as the M.A.D. by either of three suspensions derived from different colonies of the infecting strains P Ut and P B.

D. Discussion of the Results of Direct (Slide) Agglutination (p. 52)

Identification of the extra-genital strains of the patient-contact groups.—Great weight may be attached to a definite reaction shared by members of the group, since in those infrequent cases in which the reaction apparently does not actually reveal the type of the strains, the demonstration of a very close non-type-specific relationship between strains derived from persons in close contact with each other strongly suggests, and, in fact, in every case tested has been shown to be, an indication of antigenic identity.

Negative results must be accepted only after repeated tests of different suspensions.

Determination of the type of the infecting strains.—It is believed that in order to explain the discrepancies described above between the results of slide typing and absorption of agglutinins, a lack of type-specificity of certain of the absorbed sera must be assumed rather than a failure of the strains in question to absorb the agglutinins of sera prepared from strains identical with them. It is, therefore, held that, while confirmation by absorption experiments of the deductions made from strong reactions on the slide with thoroughly reliable sera is not essential, it is indispensable when these conditions are not fulfilled.

E. Direct (Water-Bath) Agglutination with Unabsorbed Sera

1. *Experiments with Sera at a* 1/32*nd-of-the-Titre Dilution* (*Charts* I *and* II)

The tests were carried out in two series : in the first series of 25 sera and their homologous strains, including all the infecting strains for which suitable suspensions were available at the time, the majority were of different serological types ; in the second series of 16 sera and their homologous strains several of the sera and strains were of the same type. Five sera and their homologous strains figure in both series.

From the figures were deleted 4 sera and their homologous suspensions which reacted poorly together and not at all with other suspensions and sera, because it seemed that the inclusion of such relatively insensitive reagents would unfairly augment the number of negative results. Those are included, however, which, although reacting poorly, or even in one instance not at all, with the homologous suspension and serum, yet showed a definite reaction with other suspensions and sera of their own or heterologous types.

Charts I and II show :—

(1) That the number of reactions occurring between sera and strains of different serological type is relatively small: 3 sera and 5 strains account for the greater number of them. The exceptional behaviour of the serum P Ut 97 (WIN 33) is discussed on p. 60.

(2) That there is little correlation between the degree of type-specificity exhibited by the sera and the suspensions of strains from which they were derived : that is, while a serum in these tests may show a high degree of type-specificity, the suspension of its homologous strain may show strong affinities with a number of heterologous sera, or, more rarely, a serum may show a lower degree of specificity than its homologous strain.

2. *Experiments with Sera at a* 1/128*th-of-the-Titre Dilution*

The tests shown in Chart II were repeated with the sera at this dilution. Little difference in the number of reactions was seen. Two

sera showed a definite zone of inhibition of agglutination at the lower dilution which masked the whole results with them.

In addition a number of tests of agglutinating suspensions of the absorbing strains performed with the untreated serum at the time of the absorption experiments gave results which differ in no way from those shown in the Charts.

3. *Discussion of the Results*

These tests have afforded important confirmatory evidence of the conclusions drawn from other techniques in the identification of strains. In addition they furnish valuable information of the serological quality of the strains and of the sera at the dilution used, as the behaviour of two sera whose reactions are described on p. 60 clearly shows.

In view of the fact that in an earlier experiment certain sera, some of which were the same as and some different from those used in these tests, put up at a dilution of 1/50 without regard to their titres showed a number of reactions with heterologous strains, the result of the experiment described under 2 above is surprising.

Further attention will be given to the reactions of sera at different dilutions.

F. Comparison of the Results of the Present Experiments with those of Andrewes and Christie (1932)

It is clear that the objectives in the two studies were essentially different, that of Andrewes and Christie being the detailed analysis of the antigenic structure of the haemolytic streptococcus, while that of the present study was to demonstrate whether or not, with the technique used, a type antigen could be shown to be shared by certain strains.

Corresponding with these differences in objective there is a notable dissimilarity in the standards of serological identity adopted in the interpretation of the results. Whereas Andrewes and Christie within the limits of their technique were frequently able to detect exhaustion of the sera up to 99 per cent., and, on the other hand, regarded a 50 per cent. reduction of titre in absorbed samples as significant for their purpose, in this study " complete absorption " seldom denotes more than a 96·8 per cent., and no account was taken of a 50 per cent., reduction of titre.

In the study of Andrewes and Christie minor degrees of absorption by heterologous strains, suggestive of some antigenic relationship short of identity between strains, were important in themselves, whereas in this study they were held to be insignificant (p. 50).

The recognition of evidence of incomplete absorption of a serum by strains believed to be identical with the homologous strains was essential in either study. In this study, with the exception of one suspension of a strain (P_5 PF (CUR 9), p. 55) no example has occurred of failure to absorb a satisfactory serum by a strain which collateral evidence had shown to be identical with the homologous strain, but

in the report of Andrewes and Christie it is difficult to detect the occurrence since the collateral evidence in the case of many of their strains appears to be lacking.

Both studies were deeply concerned with the occurrence of any high degree of absorption of a serum by heterologous strains since this, if unrecognised, will inevitably lead to the making of false deductions of identity, and must, if of sufficiently frequent occurrence, discredit the whole method of approach to the problem.

In the experience of Andrewes and Christie this disquieting occurrence was certainly more frequent than in the present work. There is reason to think that this may be associated with differences in the procedures and particularly with the fact that the sera used by Andrewes and Christie showed titres ranging from 1/325 to 1/9,000, with the majority not above 1/2,000, and that these sera were absorbed at a fixed dilution of 1/25 with absorbing doses which in many experiments were intentionally heavy and unrelated to the M.A.D. The technique, therefore, differed in certain respects believed to be important from that used in this work. The titres of a number of their sera were very little above the figure found to be the lower limit of safety in this study and many of them must have been well below that figure, while the period of immunisation is not stated. In this study (p. 60) an apparent correlation was seen in the results of the absorption experiments between a low titre—or even a somewhat higher titre obtained only by prolonged immunisation—and various anomalies, notably, irregular absorption by heterologous strains.

It would seem possible, then, that the character of the sera and of the experimental conditions may have contributed to the "chaotic" results described, and, further, that the relatively crude methods used in both these studies, though useful for the recognition of identical serological types, may be quite unsuitable for the determination of the finer details of antigenic structure. It may well be that that objective will be achieved only when the antigenic fractions isolated from the complex bacterial cell are available for serological analysis.

VII.—GENERAL SUMMARY

A serological study was made of the infecting strains of haemolytic streptococci isolated from cases of puerperal fever, and of the relationship existing between them and the extra-genital strains found in the patients and their contacts.

Strains were identified by direct (slide) agglutination with absorbed sera of known type, kindly supplied by Dr. F. Griffith; and by absorption of agglutinins of sera prepared from the strains isolated from members of the patient-contact groups, the titrations of the treated samples of sera being carried out in the water-bath. The results of these two techniques showed close agreement throughout. In addition, direct (water-bath) agglutination tests with unabsorbed sera, performed with a number of strains, on the whole gave clear-cut results which confirmed the findings of the other two methods.

The technique of slide-typing described by Griffith was used.

The technical difficulties associated with absorption experiments and with water-bath agglutination tests with these organisms have been, to a great extent, successfully overcome. Special attention has been given to the preparation of sensitive and stable agglutinating suspensions. The direct (water-bath) agglutination tests were set up at a dilution which was a constant fraction of the titre. In the absorption experiments the sera were treated with absorbing doses ascertained to be sufficient for the absorption of the serum by the homologous strain, at a dilution which was a constant fraction of the titre. By the adoption of this absorption technique, and by the use of sera of good titre, produced by a ready response in the animal body, the well-recognised liability of sera prepared from these organisms to lose their homologous agglutinins when treated with heterologous strains is diminished; the inclusion in every absorption experiment of control strains known to be of different type from the homologous strain ensures the detection of the irregularity when it occurs.

A. The Serological Type of the Infecting Strains

In a series of 121 puerperal strain isolated from cases of definite infection, all of which were derived from separate outbreaks or sporadic cases, it was found that 85 reacted with one or other of 18 of the sera prepared by Griffith from well-defined serological types isolated from human infections, chiefly from scarlet fever and tonsillitis. It is probable that insensitivity of the agglutinating suspension used accounted for the failure of the majority of the remainder of the strains to react with any of the type sera.

Eighty-five per cent. of the 85 strains typed belong to one or other of 11 types, and 54 per cent. to one or other of 5 types. Thus, the majority of puerperal strains belong to a limited number of types, and these are the types most commonly met with in other human diseases caused by haemolytic streptococci, notably in diseases of the respiratory passages. This fact, in itself, affords strong indirect evidence of the part played by the organisms of the respiratory tract in the causation of puerperal fever; it is also of importance in the study of the problems of experimental and clinical immunisation or serum therapy.

B. The Source of the Infection

The source of the infection of 67 patients was investigated.

A possible source for the infection, that is, an extra-genital strain serologically identical with the infecting strain, was found in 48 (76 per cent.) patients. Concerning the strains of 4 patients and their contacts no conclusion was reached, owing to technical difficulties in the experiments.

It is explained that, owing to failure, in the early stages of the work, to realise the importance of swabbing the nose as well as the

throat of patient and contacts, and the members of the household of the patient as well as her attendant-contacts, and owing to the difficulties experienced in securing the full complement of swabs, and to the use, in certain cases, of an ineffective swabbing technique, the figure quoted above is fallaciously low. Thus, in the 31 patient-contact groups in which the patients, and, so far as could be ascertained, all their attendant-contacts were swabbed, a possible source for the infection of 27 (87 per cent.) patients was found, whereas in the 32 patient-contact groups in which swabs were known to be missing, the percentage was only 65; in the case of 4 only of the 15 patients for whose infection no source was found was the full number of swabs received from patient and attendant-contacts, and in the case of one only of these 15 patients were swabs taken from the children of the household.

The possible source of the infection was found in the respiratory passages of the patient 24 times and in a septic focus on her skin once; in the respiratory tract of one or more of the members of the group of attendant and household contacts of the patient 36 times and in a septic focus on the skin of a child of a patient once; it was found in both patient and contact 14 times.

The strain identical with the infecting strain was found in the nose swab of the patient about as often as in her throat swab. Figures for the respective incidence in contact nose and throat swabs are of little value owing to the deficiencies in the material supplied. While experimental evidence as to the readiness with which throat strains of healthy carriers are disseminated is at present incomplete, there can be little question of the opportunities which exist for the diffusion of nose organisms by means of handkerchiefs.

In 13 of the 63 groups a strain identical with the infecting strain was found in a member of the household of the patient.

The extra-genital strains isolated from the patient and the members of her household have been designated "familial" strains, to distinguish them from "professional" strains carried by her attendants. Even when due allowance is made for the fact that some of these "familial" strains were derived from selected families, it is clear that the findings serve to emphasise the very important fact that any source of the haemolytic streptococci which are pathogenic for human beings anywhere in her environment is a potential source of danger to the patient, and that measures designed to protect her from infection by these "familial" strains conveyed by her own hand, or perhaps by towels, etc., contaminated by secretions of the nose or throat, merit the most serious attention.

A contact of 13 patients was known to have suffered from an inflammatory condition of the nose or throat shortly before the confinement: from 12 of these 13 contacts a haemolytic streptococcus was isolated which was found to be identical with the infecting strain. This finding shows the very special risk which the parturient woman runs when her confinement is conducted in an infected environment.

When strains identical with the infecting strain were found in the patient and also in one or more contacts, it was difficult, or impossible, to decide which, if any, of these was responsible for the infection. A similar difficulty was met with when it was known or suspected that not all the contacts had been swabbed effectively. A careful study of the combined serological and epidemiological evidence, however, appears to justify the assumption that of the 48 patients for whose infection a possible source was found, 6 did actually contract their infection from their own extra-genital strain, 24 from an attendant-contact strain and 9 from a strain found in a member of the household of the patient. In the case of 9 patients there was nothing in the epidemiological evidence on which to decide the point.

By a calculation based on the experimental evidence actually obtained with the present admittedly imperfect procedures and on the comparable figures already published by J. Smith, it is estimated that the deaths of at least 576 women and the non-fatal illness of at least 2,304 women, in addition, might be avoided annually in England and Wales if infections due to haemolytic streptococci were prevented.

The results of recent research are quoted to show that the haemolytic streptococci which are pathogenic for human beings may be differentiated from other haemolytic streptococci by serological and biochemical tests; that the organisms which cause puerperal fever are not to be found in the vagina at the beginning of labour; and that the most important sources of such organisms are in the human respiratory passages and in septic foci. Acceptance of these facts leads to the confident expectation that collaboration between clinicians and bacteriologists will result in the development of methods which will prevent the entry of these organisms to the genital tract during labour and the early days of the puerperium in all but exceptional circumstances. Reference is made to experimental evidence which points the way to this end, and clinical evidence is cited to show that already in certain maternity institutions that task has been brought near to achievement.

VIII.—ACKNOWLEDGMENTS

My very grateful thanks are due to Dr. F. Griffith for prolonged instruction in his method and for his most generous gift of absorbed sera, without which the scope of this study would have been greatly restricted.

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X.—APPENDIX

(a) *Glucose phosphate broth.*

Minced beef	500 gms.
Water	1,000 c.c.
Proteose peptone	2 per cent.
Glucose	0·2 ,,
Na_2HPO_4	0·2 ,,

p H 7·6

(b) *Trypsin phosphate broth.*

Minced beef	400 gms.
Water	1,000 c.c.
Na_2HPO_4	1 gm.
Na Cl	0·25 per cent.

p H 7·6

(c) *Blood agar plates.*

Digest-ox-heart-broth Powdered agar (2½ per cent.)	92 per cent.
Horse blood	8 per cent.

TABLE I

The details of the absorption experiments and the results of direct (slide) agglutination tests with the strains of the patient-contact groups

Explanation of Table I

Abbreviations used :—

P_1, P_2, etc. = patients.
N_1, N_2, etc. = nurses.
D_1, D_2, etc. = doctors.
Hw = the handywoman.
Ch_1, Ch_2, etc. = children of the patient.
H = husband of the patient.
Ut = strain isolated from the uterus.
B = ,, ,, ,, blood.
PF = ,, ,, ,, peritoneal fluid.
Pl F = ,, ,, ,, pleural fluid.
T = ,, ,, ,, throat.
N = ,, ,, ,, nose.
G = ,, ,, ,, gums.
Ps = ,, ,, pus.

The designations of the sera, strains and patient-contact groups are as follows :—

P_1 Ut 80 (*BRI* 4) indicates a serum, number 80, prepared from the uterine strain of the patient, number 1, in the patient-contact group (BRI 4).

The letters a and b, following a serum number, indicate specimens of sera obtained by bleedings of the same rabbit at intervals of some weeks: for the purposes of the absorption experiments thay are counted as different sera.

The Griffith type sera and strains have the numbers allocated to them by Dr. Griffith (1935) preceded by the letters " FG ".

Columns (2) " T.N.F." = type not found.
(3) Strains not indicated were not tested.
(4) " ? " denotes an indefinite reaction.
Column (5) " Rep " = repetition of an experiment with the same serum as before.
(8) The smallest absorbing dose used is the M.A.D.
" > M.A.D. " = an unmeasured absorbing dose larger than, and probably considerably larger than, the M.A.D.
Columns (10) to (14).
" ? " = a result which was difficult to interpret on account of reduction of the volume of the agglutinate in each tube of the treated sample (p. 50).

The " control strains " (p. 14) used in the experiments with the sera of a patient-contact group are designated C_1, C_2 and C_3 in order to show to what extent consistent results were obtained with the same strain in repeated experiments with the sera of a patient-contact gp. The use of the same letters in the experiments with the sera of other gps does not mean that the control strains were the same.

When, for any reason, it is of interest to know it, the type of the control strain is denoted in Column 15.

The character of the results

The clue to the character of the results obtained in the experiments is given by a glance at Columns (9) to (14). All strains in Column (10) should effect the same degree of absorption as the homologous strain, if not at the M.A.D., at a dose not more than three times as great (p. 14) as the M.A.D., while no strain in Column (12) should effect more than a 50 per cent.—or, at most, a 75 per cent. (p. 59)—reduction of titre. A repetition of an experiment shows the significance of a partial absorption.

When the absorption experiments with a strain gave contradictory results or results which did not accord with other evidence of identification, and when there was no reason to impugn the validity of the one or the other technique, the strain appears in Column (14). Thus, in certain of the absorption experiments with the serum *P Ut* 44 (*WOO* 34) the strains C_2 [P B (NAS 18)] and D T (WOO 34) both absorbed 93·75 per cent. Since there is abundant collateral evidence to show that the first strain is not identical with the strain P Ut (WOO 34) it figures in Column (12) as a heterologous strain, while since, on the other hand, no conclusion was reached as to the identity of the strain D T (WOO 34) it appears in Column (14).

TABLE I

Patient-contact group.	Direct agglutination on the slide.			Absorption experiments.											Notes.
								Degree of absorption effected by							
	Type of infecting strain.	Strains of the gp reacting with the type serum.	Strains of the gp not reacting with the type serum.	Serum.	Titre.	Serum dilution in absorbing mixtures (fraction of litre).	Absorbing dose in millions per c.c	The homologous strain.	Strains regarded as serologically identical with the homologous strain.		Strains regarded as serologically different from the homologous strain.		Strains whose relationship to the homologous strain was not determined.	% absorbed.	
									Strain.	% absorbed.	Strain.	% absorbed.			
Col. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)		(15) Cols.
ARM 1	TNF			*P_1 Ut 47*	3,200	1/100 (1/32)	10,000	96·8	P_2 Ut P_1 T P_2 T	96·8 96·8 96·8	C_1	<50			
				Rep	1,600	1/100 (1/16)	10,000	93·75	P_2 Ut P_1 T	93·75 93·75	C_2	<50			
BIN 1a	? FG 4 ? FG 19 (p. 63)	P B P Ut		*P B 99*	25,600	1/1600 (1/16)	40,000	93·75	P Ut	93·75	D_1 T C_1 C_2	<50 ,, ,,			C_6 = Strain FG 4. C_3, C_5, C_7 = Infecting strains of other gps of type FG 4. C_8 = Strain FG 19. P Ut (1), ,, (2) = Different absorbing suspensions of the strain P Ut.
	For reciprocal absorption experiment with the serum FG 4 see end of this Table.			Rep	25,600	1/800 (1/32)	40,000	96·8	P Ut (1) P Ut (2)	96·8 96·8	C_3 C_4 C_5 C_6 C_7 C_8	<50 50 ,, <50 ,, ,,			
BIR 2	TNF			*P B 51*	25,600	1/400 (1/64)	20,000	98·4			N_1 T N_2 T C_1	<50 ,, ,,			
BOD 3	FG 17	P Ut Ch_1 T Ch_2 T Ch_3 T		*P Ut 70a*	38,400	1/600 (1/64)	40,000	96·8	Ch_1 T Ch_2 T	96·8 96·8	C_1 C_2	50 ,,			
				70b	4,800	1/150 (1/32)	40,000	96·8			P G C_3 C_4	<50 ,, ,,			
				Ch_1 E 82	12,800	1/400 (1/32)	20,000	96·8	P Ut Ch_1 T	96·8 96·8	C_3 C_4 C_5	<50 50 <50			

BRI 4	FG 11	P_1 Ut P_2 B D_1 T P_2 T_1	P_2 T_2 D_2 T	*P_1 Ut 80*	1,600	1/100 (1/16)	40,000	93·75	P_2 B D_1 T	93·75 93·75	C_3 C_5	<50 ,,			
				Rep	1,600	1/50 (1/32)	40,000	96·8	P_2 B D_1 T C_1	96·8 96·8 96·8					C_1 = P Ut (LUF 16).
				Rep	800	1/50 (1/16)	40,000	93·75			C_2 C_3 C_4	<50 ,, ,,			
				P_2 B 64	800	1/50 (1/16)	12,000	93·75	P_1 Ut	93·75	P_2 N C_5 C_6	50 <50 ,,			
				Rep	1,600	1/100 (1/16)	20,000	93·75	P_1 Ut	93·75	P_2 N D_2 T C_8	<50 ,, 75			
				Rep	1,600	1/50 (1/32)	40,000	96·8	P_2 T_1	96·8	P_2 T_2 D_2 T C_7	<50 ,, 75			
				P_2 T_1 74	3,200	1/200 (1/16)	6,000	93·75	P_2 B	93·75	P_2 T_2 C_8 C_7	? 90 75 ,,			
				Rep	3,200	1/200 (1/16)	20,000	93·75	P_2 B	93·75	D_2 T C_7 C_9 C_8	93·75 ?93·75 ,, <50			
				Rep	3,200	1/200 (1/16)	20,000	93·75	P_2 B	93·75	C_2 C_5 C_{10}	?93·75 ,, ,,			
				Rep	6,400	1/200 (1/32)	20,000	96·8	P_1 Ut P_2 B	96·8 96·8	P_2 T_2 D_2 T	93·75 ,,			
BUC 4a	FG 1	P_1 Ut P_2 Ut P_3 Ut N_1 T_1 N_1 T_2 N_2 T P_1 B P_1 T P_2 T HP_2 T HP_2 N		*P_1 Ut 102*	6,400	1/200 (1/32)	60,000	96·8	P_2 Ut N_1 T_1 N_1 T_2	96·8 96·8 96·8	C_1 C_2	<50 ,,			
				P_2 Ut 101	1,600	1/100 (1/16)	80,000	93·75	P_1 Ut P_3 Ut N_1 T_2	93·75 93·75 93·75	C_3 C_4	<50 50			
				N_1 T_2 100	25,600	1/1600 (1/16)	80,000	93·75	P_1 Ut P_2 Ut N_1 T_1	93·75 93·75 93·75	C_5 C_6	75 <50			

TABLE I (*contd.*)

Patient-contact group.	Direct agglutination on the slide.			Absorption experiments.											Notes.
								Degree of absorption effected by							
									Strains regarded as serologically identical with the homologous strain.		Strains regarded as serologically different from the homologous strain.		Strains whose relationship to the homologous strain was not determined.	% absorbed.	
	Type of infecting strain.	Strains of the gp. reacting with the type serum.	Strains of the gp. not reacting with the type serum.	Serum.	Titre.	Serum dilution in absorbing mixtures (fraction of litre).	Absorbing dose in millions per c.c.	The homologous strain.	Strain.	% absorbed.	Strain.	% absorbed.			
Col. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)		(15) Cols.
BUS 5	FG 13	P Ut Ch T N_1 T	N_2 T N_4 T	*P Ut 21*	1,600	1/25 (1/64)	40,000	98·4	Ch T N_1 T N_1 T	98·4 98·4 98·4	C_1 C_2	<50 ,,			C_5 = Strain FG 13.
				Ch T 29	1,024	1/32 (1/32)	> M.A.D.	96·8	P Ut N_1 T N_2 T C_5	96·8 96·8 96·8 96·8	C_3 C_4 C_6 C_7	<50 ,, 75 ,,			
				N_1 *T 28*	12,800	1/50 (1/256)	> M.A.D.	99·6	P Ut	99·6	C_1	<50			
BYR 6	TNF			*P Ut 41*	640	1/25 (1/32)	40,000	96·8					P T H Ps C_1 C_2	75 96·8 96·8 93·75	H Ps = Pus from septic hand of husband.
CHA 7	FG 1	P Ut P N Hw T		*P Ut 92*	12,800	1/400 (1/32)	60,000	96·8	P N Hw T	96·8 96·8	P G C_1 C_2	<50 ,, ,,			C_3 = Strain FG 1. C_5 = P B ((D) TA 27). C_6 = P Ut (WAR 32). C_7 = P_1 Ut (BUC 4a).
				Rep	12,800	1/400 (1/32)	60,000	96·8	C_3 C_5 C_6 C_7	96·8 96·8 96·8 96·8	C_1 C_4	<50 ,,			
CRO 8	FG 2	P Ut N T	Hw T	*P Ut 96*	3,200	1/100 (1/32)	80,000	96·8	N T	96·8	Hw T C_1	<50 ,,			

CUR 9	FG 12	P_1 Ut P_4 Ut P_5 PF N_1 T N_2 T	N_2 T	*P_1 Ut 76*	2,400	1/75 (1/32)	40,000	96·8	P_1 T P_2 T P_3 Ut P_5 PF	96·8 96·8 96·8 96·8	C_1 C_2	<50 ,,			For a note on the personnel of this gp see Table IV.
				Rep	2,400	1/75 (1/32)	40,000	96·8	P_4 Ut P_5 PF	96·8 96·8	N_2 T C_1	<50 ,,			For comments on the results of direct agglutination on the slide, see p. 63.
	FG 10	P_4 Ut P_3 Ut N_5 NT N_6 T N_7 T	N_8 T N_9 T P_5 PF N_1 T P_1 Ut	Rep	2,400	1/75 (1/32)	40,000	96·8	N_1 T	96·8	C_3	<50			C_1 = Strain FG 10.
	For reciprocal absorption experiment with the serum FG 10 see end of this Table.			*P_4 Ut 63*	1,600	1/100 (1/16)	6,000	93·75	P_6 Ut P_7 T P_8 T N_1 T N_2 T N_4 T	93·75 93·75 93·75 93·75 93·75 93·75	N_2 T C_2 C_5 C_9	75 ,, ,, ,,			
				P_4 Ut 77	3,200	1/200 (1/16)	40,000	93·75	N_2 T P_5 PF	93·75 93·75	N_3 T C_2	<50 ,,			
				Rep	3,200	1/100 (1/32)	40,000	96·8	P_1 Ut P_5 PF	96·8 96·8	C_5	<50			
				N_2 T 75a	1,600	1/100 (1/16)	30,000	93·75	P_4 Ut P_5 PF	93·75 ?<50	N_3 T C_3	<50 ? ,,			
				75b	3,200	1/100 (1/32)	40,000	96·8	P_4 Ut P_5 PF	96·8 96·8	C_2	<50			
				Rep	3,200	1/100 (1/32)	40,000	96·8			C_2 C_3 C_4	<50 ,, ,,			
				P_5 PF 56	1,600	1/100 (1/16)	2,500	93·75	N_1 T	93·75	C_5 C_7	93·75 ,,			
				Rep	320	1/20 (1/16)	3,500	93·75	N_1 T	93·75	C_5 C_6 C_7	<50 ,, ,,			
				P_5 PF 84	1,600	1/50 (1/32)	10,000	96·8	N_1 T P_4 Ut	96·8 96·8	C_2 C_8	<50 ,,			
				Rep	1,600	1/50 (1/32)	10,000	96·8	P_1 Ut	96·8	C_5 C_7 C_{11}	<50 ,, ,,			

TABLE I (*contd.*)

Patient-contact group.	Direct agglutination on the slide.			Absorption experiments.											Notes.
								Degree of absorption effected by							
	Type of infecting strain.	Strains of the gp. reacting with the type serum.	Strains of the gp. not reacting with the type serum.	Serum.	Titre.	Serum dilution in absorbing mixtures (fraction of titre).	Absorbing dose in millions per c.c.	The homologous strain.	Strains regarded as serologically identical with the homologous strain.		Strains regarded as serologically different from the homologous strain.		Strains whose relationship to the homologous strain was not determined.	% absorbed.	
									Strain.	% absorbed.	Strain.	% absorbed.			
Col. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)		(15) Cols.
CUR 9 (*cont.*)				N_1 T 55a	1,600	1/50 (1/32)	6,000	96·8	N_2 T P_4 Ut P_5 PF (6/7)	96·8 75·00 50·0	C_3 C_{10}	<50 "			P_5 PF (6/7) = Absorbing suspension, dated 6/7/33.
				Rep	1,600	1/50 (1/32)	20,000	96·8	P_4 Ut P_5 PF (6/7)	96·8 <50	C_3 C_{10}	<50 "			
				Rep	800	1/50 (1/16)	20,000	93·75	P_5 PF (21/10)	93·75	C_2 C_8	<50 50			P_5 PF (21/10) = Absorbing suspension, dated 21/10/33.
				55b	3,200	1/100 (1/32)	15,000	96·8	P_1 Ut P_4 Ut P_5 PF (21/10)	96·8 ?87·5 ?87·5	C_1 C_3 C_4	75 <50 "			
				N_1 T 87	6,400	1/100 (1/64)	32,000	98·4	P_1 Ut P_4 Ut P_5 PF	98·4 98·4 98·4	C_{12}	<50			
				Rep	6,400	1/100 (1/64)	32,000	98·4	P_1 Ut P_4 Ut	98·4 98·4	C_1 C_1 C_{12} C_{12} C_{13}	<50 " " " "			
				Rep	3,200	1/100 (1/32)	33,000	96·8	P_5 PF	96·8	C_{14}	<50			
EAS 10	FG27	P Ut P PF		P Ut 58	2,560	1/80 (1/32)	2,500	96·8	P PF	96·8	C_1 C_2	<50 "			No investigation of the source of the infection of this patient was made: P_5 PF = a strain showing beta-haemolysis *only under anaerobic conditions.*
	For reciprocal absorption experiment with the serum FG 27 see end of this Table.			P PF 59	800	1/50 (1/16)	2,000	93·75	P Ut	<50	C_1 C_2	<50 93·75			
				Rep	800	1/50 (1/16)	2,000	93·75	P Ut	<50	C_1 C_2 C_3 C_4	<50 93·75 75 93·75			
HAL 11	TNF			P Ut 60	6,400	1/200 (1/32)	5,000	96·8			N T C_1 C_2	<50 " "			
				Rep	6,400	1/200 (1/32)	13,000	96·8			N T C_1	<50 "			
HAR 12	FG 11	P Ut N_1 T		P_1 Ut 25	7,680	1/240 (1/32)	> M.A.D.	96·8	P_2 Ut P_3Pl F N_1 T N_1 T N_2 T P_3 T	96·8 96·8 96·8 96·8 96·8 87·5	C_1 C_2 C_3	<50 50 "			C_5 = Strain FG 11. C_7 = Strain P Ut (LUF 16). C_8 = Strain P B (BRI 4). For comment on the results see p. 63.
				Rep	12,800	1/128 (1/64)	60,000	98·4	N_1 T	98·4	C_5 C_6 C_7 C_8 C_9	<50 75 " 50 75			
				N_1 T 33	3,200	1/100 (1/32)	M.A.D.	96·8	P_1 Ut P_2 Ut	96·8 96·8	C_4	<50			
HAY 13	FG 17	P Ut P N N N		P Ut 52	25,600	1/400 (1/64)	3,000	98·4	P N N N C_1	87·5 98·4 87·5	C_2 C_3	<50 "			C_1 = Strain FG 17.
				Rep	25,600	1/400 (1/64)	6,000	98·4	P N C_1	87·5 93·75	C_2	<50			
				Rep	25,600	1/800 (1/32)	5,000	96·8	P N N N N T C_1	93·75 96·8 96·8 93·75	C_3	<50			
				Rep	6,400	1/400 (1/16)	30,000	93·75	N N N T C_4	93·75 93·75 93·75	C_5	<50			C_4 = Strain P Ut (TRI 30) of type FG 15 (p. 52).
				N T 54	102,400	1/1600 (1/64)	3,000	98·4	P Ut N N P N C_1	98·4 98·4 93·75 93·75	C_2 C_3	<50 "			
				Rep	102,400	1/1600 (1/64)	6,000	98·4	P N C_1	98·4 98·4	C_2 C_3	<50 "			

TABLE I (*contd.*)

Patient-contact group.	Direct agglutination on the slide.			Absorption experiments.											Notes.
								Degree of absorption effected by							
	Type of infecting strain.	Strains of the gp reacting with the type serum.	Strains of the gp not reacting with the type serum.	Serum.	Titre.	Serum dilution in absorbing mixtures (fraction of titre).	Absorbing dose in millions per c.c.	The homologous strain.	Strains regarded as serologically identical with the homologous strain.		Strains regarded as serologically different from the homologous strain.		Strains whose relationship to the homologous strain was not determined.	% absorbed.	
									Strain.	% absorbed.	Strain.	% absorbed.			
Col. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)		(15) Cols.
LAW 14	FG 6	P Ut P N		*P Ut 34*	6,400	1/200 (1/32)	12,000	96·8	P N C_1	96·8 96·8	C_2 C_3	<50 "			C_1 = Strain FG 6.
				Rep	6,400	1/200 (1/32)	16,000	96·8	P N C_1	96·8 93·75	C_3 C_4 C_5	<50 " "			
				P N 43	3,200	1/100 (1/32)	6,000	96·8	P Ut C_1	96·8 93·75	C_4 C_5	<50 "			
LEC 15	TNF			*P Ut 94*	3,200	1/100 (1/32)	60,000	96·8	P PF P N N T	96·8 96·8 96·8	C_1 C_2 C_3	<50 " "			
LUF 16	FG 11	P Ut P B P V P PC		*P Ut 67*	12,800	1/100 (1/128)	60,000	96·8	P PC P B P V	96·8 99·2 99·2	C_1 C_2	<50 "			The strains isolated from the pericardium (P PC) and a thrombosed vein (P V) showed beta-haemolysis *only under anaerobic conditions*; the strain isolated from the blood (P B) and the uterus (P Ut) showed respectively slight and good haemolysis in aerobic culture.
				Rep	6,400	1/400 (1/16)	40,000	93·75	P PC P N	93·75 93·75	C_3	<50			
				P PC 68	12,800	1/800 (1/16)	20,000	93·75	P Ut	93·75	C_2 C_4	<50 "			
				Rep	12,800	1/400 (1/32)	20,000	96·8	P B P V P N	96·8 96·8 93·75	C_2 C_5	<50 "			
				Rep	25,600	1/400 (1/64)	40,000	98·4	P Ut P N	98·4 98·4	C_2	<50			
				P N 78	1,600	1/100 (1/16)	10,000	93·75	P Ut P PC C_8	93·75 93·75 75·00	C_6 C_7	<50 "			C_8 = Strain FG 11.
				Rep	1,600	1/100 (1/16)	20,000	93·75	P PC C_8	93·75 93·75	C_9	<50			
MAR 17	TNF			*P B 39*	12,800	1/400 (1/32)	5,000	96·8			Ch_1 T C_1 C_2 C_3 C_4 C_5	<50 " " " " "			
				Rep	6,400	1/400 (1/16)	10,000	93·75			Ch_1 T Ch_2 T Hw T	<50 " "			
				Rep	6,400	1/400 (1/16)	20,000	93·75			Ch_1 T Ch_2 T	<50 "			
NAS 18	?FG 16	P B P N D T	P T	*P B 32*	25,600	1/800 (1/32)	1,000	96·8			C_1 C_2 C_4	<50 " "	D T	75	*For comments on these results see p. 57.*
		N_1 T		Rep	102,400	1/800 (1/128) (1/16)	2,000	99·2			C_3	50	D T D T D T P N C_5	50 <50 " " "	C_5 = Strain FG 16.
				Rep	51,200	1/1600 (1/32)	1,000	96·8			C_4	<50	P N D T	63 50	
				Rep	25,600	1/800 (1/32)	50,000 or 100,000	96·8			P T N_2 T C_6 C_7 C_8	<50 " " " "	P N D T N_1 T Hw T C_5	96·8 96·8 96·8 96·8 96·8	Experiments performed Jan.–June, 1933.
				N_1 T 49	3,200	1/100 (1/32)	4,000	93·75					P B D T P N	<50 " "	
				D T 50	6,400	1/100 (1/64)	8,000	98·4			C_1	<50	P B P N N T	<50 98·4 <50	
				Rep	6,400	1/100 (1/64)	8,000	98·4					C_5 P N	<50 98·4	

TABLE I (*contd.*)

Patient-contact group.	Direct agglutination on the slide.			Absorption experiments.											Notes.
	Type of infecting strain.	Strains of the gp re-acting with the type serum.	Strains of the gp not re-acting with the type serum.	Serum.	Titre.	Serum dilution in absorbing mixtures (fraction of titre).	Absorbing dose in millions per c.c.	Degree of absorption effected by: The homologous strain.	Strains regarded as serologically identical with the homologous strain. Strain.	% absorbed.	Strains regarded as serologically different from the homologous strain. Strain.	% absorbed.	Strains whose relationship to the homologous strain was not determined.	% absorbed.	
Col. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)		(15) Cols.
NAS 18 (*cont.*)				*P B 32*	25,600	1/800 (1/32)	5,000	96·8			C_9 C_{10} C_2	<50 " "	D T	50	Experiments performed October, 1934.
				Rep	25,600	1/800 (1/32)	80,000	96·8			C_9 C_2	50 <50	D T C_5	65 ?75	
				Rep	51,200	1/1600 (1/32)	20,000	96·8			C_2 C_9	<50 "	D T C_5	<50 "	Experiments performed with another agglutinating suspension of the strain P B.
				Rep	51,200	1/3200 (1/16)	120,000	93·75			C_2 C_9	<50 50	D T C_5	80 75	
				D T 50	3,200	1/200 (1/16)	60,000	93·75			C_{12} C_{13}	<50 "	P B C_5	<50 "	
OAK 19	FG 25	P_1 Ut D T P_3 Ut P_2 N		*P_1 Ut 65*	12,800	1/800) (1/16)	3,000	93·75	P_1 B P_3 Ut P_3 N D T	93·75 93·75 93·75 93·75	N_1 T N_2 T C_1 C_4	<50 " " "			
				Rep	12,800	1/800 (1/16)	6,000	93·75	D T	93·75	C_1	<50			
				D T 69	12,800	1/400 (1/32)	20,000	96·8	P_1 Ut P_1 B P_2 Ut P_2 N	96·8 96·8 96·8 96·8	C_2 C_3 C_5	<50 " "			

PAG 20	FG 2	P B P Ut P T_1	P T_2	*P B 62*	51,200	1/800 (1/64)	10,000	98·4	P Ut P N P T_1	98·4 98·4 98·4	P G P T_2 C_4	<50 " "			Patient's throat yielded 2 strains of different colonial appearance (P T_1 and P T_2).
				P N 79	102,400	1/1600 (1/64)	10,000	98·4	P Ut C_3	87·5 98·4	C_1 C_2	<50 "			C_3 = Strain FG 2.
				Rep	102,400	1/1600 (1/64)	20,000	98·4	P Ut	98·4	C_2	<50			
PIC 21	FG 13	P B P T N T C_4 Ps P N		*P B 23*	1,600	1/100 (1/16)	> M.A.D.	93·75	P T N T C_2 C_4 Ps C_4 Ps	93·75 93·75 93·75 93·75 93·75	C_1 C_3 C_6	<50 " "			C_2 = Strain FG 13. C_4 Ps = Strain from the septic hand contracted by the nurse who attended this patient after the onset of the infection.
				N T 24	3,200	1/50 (1/64)	> M.A.D.	98·4	P B	98·4	C_6 C_7 C_8	<50 " "			
				Rep	3,200	1/50 (1/64)	> M.A.D.	98·4	P B	98·4	C_6 C_7 C_8	<50 " "			
POP 22	FG 14	P Ut P N P T B_1 PF B_2 PF D T N T		*P Ut 93*	6,400	1/200 (1/32)	40,000	96·8	B_2 PF D T C_1 (a)	96·8 96·8 ? 50	C_3 C_5 C_1	<50 " "			B_1 = Baby 1 } p. 19. B_2 = Baby 2 } p. 19. C_1(a) } = 2 different absorbing suspension C_1(b) } of Strain FG 14.
				Rep	6,400	1/200 (1/32)	60,000	96·8	B_1 PF C_1 (a) C_1 (b) C_8	96·8 87·5 75·0 96·8	C_5 C_6 C_7	<50 " "			C_8 = P Ut ((R) TA 28).
				B_1 PF 95	6,400	1/200 (1/32)	40,000	96·8	P Ut B_2 PF D T N T C_1 (a)	96·8 96·8 96·8 96·8 87·5	C_3 C_5	<50 "			
SAU 23	FG 1	P Ut	P T P G N T	*P Ut 57*	6,400	1/400 (1/16)	12,000	93·75	C_1	93·75	P T P G N T C_2	<50 " " "			C_1 = Strain FG 1.
SEA 23a	FG 5	P Ut		*P Ut 105*	1,600	1/50 (1/32)	120,000	96·8			C_1 C_2 C_3	93·75 50 "	N_1 T N_2 T	93·75 87·5	C_1 C_2 C_3 } = Strains of types other than FG 5.
SMI 24	FG 17	P_1 Ut P_2 PF N_1 T P_1 PF	N_2 T N_3 T N_4 T	*P_1 Ut 90*	25,600	1/800 (1/32)	80,000	96·8	P_2 PF N_1 T	96·8 96·8	N_2 T P_1 T C_2	<50 " "			
				P_2 PF 89	12,800	1/200 (1/64)	80,000	98·4	P_1 PF C_1	98·4 98·4	P_1 T	<50			C_1 = Strain FG 17.

TABLE I (*contd.*)

Patient-contact group.	Direct agglutination on the slide.			Absorption experiments.											Notes.
								Degree of absorption effected by							
								Strains regarded as serologically identical with the homologous strain.		Strains regarded as serologically different from the homologous strain.		Strains whose relationship to the homologous strain was not determined.	% absorbed.		
	Type of infecting strain.	Strains of the gp reacting with the type serum.	Strains of the gp not reacting with the type serum.	Serum.	Titre.	Serum dilution in absorbing mixtures (fraction of titre).	Absorbing dose in millions per c.c.	The homologous strain.	Strain.	% absorbed.	Strain.	% absorbed.			
Col. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)		(15) Cols.
SOP 25	TNF			*P Ut 46*	1,280	1/40 (1/32)	2,000	96·8			P T D T C_1 C_2	<50 " "			
				Rep	1,600	1/50 (1/32)	2,500	96·8			C_3 C_4	<50 "			
SPR 26	TNF			*P Ut 42*	320	1/10 (1/32)	20,000	96·8					Ch Ps C_1 C_2	75 75 75	Ps = Pus from impetigo. For comment on this experiment see p. 55.
(D) TA 27	FG 1	P B D T		*P B 22*	51,200	1/200 (1/256)	> M.A.D.	99·6	D T C_1	99·6 99·6	C_2	<50			C_1 = Strain FG 1. C_2 = Strain P Ut (SAU 23). C_3 = Strain P Ut (WAR 32).
				D T 18	6,400	1/50 (1/128)	> M.A.D.	99·2	P B C_1	99·2 99·2	C_2 C_3	<50 "			
				Rep	51,200	1/800 (1/64)	10,000	98·4	P B C_1 C_3 C_6	99·2 99·2 99·2 99·2	C_3 C_4	<50 "			
(R) TA 28	FG 14	P Ut N T		*P Ut 71*	3,200	1/50 (1/64)	10,000	98·4	N T	98·4	C_1 C_2	<50 "			
				N T 83	12,800	1/400 (1/32)	40,000	96·8	P Ut	96·8	C_3 C_4	<50 "			
				Rep	25,600	1/800 (1/32)	40,000	93·75	P Ut	96·8	C_3 C_5	<50 "			
TEV 28a	FG 3	P Ut		*P Ut 98*	3,200	1/100 (1/32)	40,000	96·8	P N N_1 T C_1 C_3 C_5	96·8 96·8 96·8 96·8 96·8	C_2 C_4	<50 "			C_1 = Strain of type FG 3. C_3 = Strain P Ut (VEN 31a). C_5 = Strain D T (VEN 31a).
TIP 29	TNF			*P Ut 37*	6,400	1/100 (1/64)	20,000	98·4	P T	98·4	C_1 C_2	<50 "			
				P T 38	6,400	1/100 (1/64)	20,000	98·4	P Ut	98·4	C_3 C_4	<50 "			
TRI 30	FG 15	P Ut P B P G P N N_1 T N_1 N N_2 T M T P T	D T	*P Ut 81*	3,200	1/100 (1/32)	20,000	96·8	P N N_1 T	96·8 93·75	C_1 C_2	<50 "			C_4 = Strain FG 15.
				Rep	3,200	1/100 (1/32)	40,000	96·8	N_1 T M T C_4	96·8 96·8 96·8	C_3 D T	<50 "			M = Maid.
VAN 31	TNF			*P_1 Ut 31*	3,200	1/200 (1/16)	> M.A.D.	93·75	P_2 Ps	93·75	N T C_1 C_2	75 50 <50			P_2 Ps = Pus from wrist abscess.
				N T 30	6,400	1/100 (1/64)	> M.A.D.	98·4			P_1 Ut P_2 Ut C_3	<50 " "			
VEN 31a	FG 3	P Ut D_1 T	D_2 T	As the serum prepared from the strain P Ut (VEN) was not satisfactory, the strains of this group, P Ut and D_1 T, were tested in the experiment with the serum P Ut 98 (TEV 28a) (q.v.).											
WAR 32	FG 1	P Ut P B	N_4 T N_5 T	*P Ut 45*	25,600	1/800 (1/32)	2,500	96·8	P B P T N_1 T	96·8 93·75 87·5	N_2 T N_3 T N_4 T N_5 T N_6 T	<50 " " " "			C_1 = Strain FG 1.
				Rep	25,600	1/800 (1/32)	5,000	96·8	P B P T N_1 T C_1	96·8 96·8 93·75 96·8	N_3 T N_4 T C_2	<50 " "			
				Rep	25,600	1/800 (1/32)	10,000	96·8	N_1 T	96·8	N_3 T C_2	<50 "			
				N_1 T 36	3,200	1/100 (1/32)	30,000	96·8	P Ut C_1	96·8 96·8	C_2 C_3	<50 "			
				Rep	3,200	1/100 (1/32)	20,000	96·8	P Ut P Ut C_1 C_1	96·8 96·8 93·75 93·75					
WHI 33	TNF			*P Ut 35*	6,400	1/200 (1/32)	8,000	96·8	P N	96·8	C_1 C_2	75 <50			
				Rep	12,800	1/200 (1/64)	10,000	98·4	P N	98·4	C_1 C_3	<50 75			
				P N 36	3,200	1/100 (1/32)	10,000	96·8	P Ut	96·8	C_1 C_3	<50 "			

TABLE I (*contd.*)

Patient-contact group.	Direct agglutination on the slide.			Absorption experiments.											Notes.
	Type of infecting strain.	Strains of the gp reacting with the type serum.	Strains of the gp. not reacting with the type serum.	Serum.	Titre.	Serum dilution in absorbing mixtures (fraction of titre).	Absorbing dose in millions per cc.	Degree of absorption effected by: The homologous strain.	Strains regarded as serologically identical with the homologous strain. Strain.	% absorbed.	Strains regarded as serologically different from the homologous strain. Strain.	% absorbed.	Strains whose relationship to the homologous strain was not determined.	% absorbed.	
Col. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)		(15) Cols.
WIN 33a	? FG 27 P Ut For reciprocal absorption experiment with serum FG 27, see end of this Table.			*P Ut 97*	400	1/25 (1/16)	40,000	<50			C_3	50	C_1 C_2	93·75 93·75	C_1 = Strain P Ut ((R) TA 28). C_2 = Strain P Ut (WOO 34). C_3 = Strain B_1 PF (POP 22). The respiratory tract strains of this gp were not tested.
WOO 34	FG 2	P Ut P T P G	D T	*P Ut 44*	102,400	1/1,600 (1/64)	5,000	98·4	P T C_3	98·4 98·4	C_2 C_1	93·75 <50	D T	93·75	*For comments on these experiments see p. 56.*
				Rep	102,400	1/1,600 (1/64)	2,500	98·4	P T C_3	98·4 98·4	C_2	93·75	D T	93·75	C_2 = Strain P B (NAS 18). C_3 = Strain FG 2.
				Rep	102,400	1/1,600 (1/64)	10,000	98·4			C_2	93·75	D T	93·75	Experiments performed October, 1932.
				Rep	102,400	1/1,600 (1/64)	50,000	98·4			C_2	93·75	D T	93·75	*The strain D T had died before the experiments were repeated.*
				Rep	51,200	1/3,200 (1/16)	2,500	93·75	P T C_3	93·75 93·75	C_4	<50			Experiment performed June, 1933.
				Rep	51,200	1/800 (1/16)	6,000	87·5	P T	87·5	C_2 C_{12}	87·5 <50			Experiments performed November, 1933.
				Rep	6,400	1/800 (1/8)	6,000	87·5	P T	87·5	C_2 C_{13} C_{14}	87·5 <50 "			
				Rep	12,800	1/200 (1/64)	20,000	98·4	C_8	93·75	C_2 C_7 C_9 C_{10}	? 50 " " <50			C_8 = Strain P Ut (CRO 8). Experiments performed August, 1934.
				Rep	12,800	1/400 (1/32)	40,000	96·8	C_8	96·8	C_{11} C_2	? 50 "			
				Rep	12,800	1/400 (1/32)	40,000	96·8	P T	96·8	C_2 C_{15}	<50 "			
				P T 85	6,400	1/200 (1/32)	20,000	96·8	P Ut C_6	96·8 96·8	C_2 C_5	<50 "			C_6 = Strain P B (PAG 20).

TABLE I (*contd.*) *Experiments with 3 of the Griffith sera* (*p.* 62)

Patient-contact group.	Direct agglutination on the slide.			Absorption experiments.											Notes.
	Type serum.	Strains reacting with the type serum.	Strains not reacting with the type serum.	Serum.	Titre.	Serum dilution in absorbing mixtures (fraction of titre).	Absorbing dose in millions per c.c.	Degree of absorption effected by: The homologous strain.	Strains regarded as serologically identical with the homologous strain. Strain.	% absorbed.	Strains regarded as serologically different from the homologous strain. Strain.	% absorbed.	Strains whose relationship to the homologous strain was not determined.	% absorbed.	
Col. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)		(15) Cols.
—	FG 4	P Ut (BIN 1a) P B (BIN 1a) C_1 C_5 C_7	C_3 C_6	*FG 4 (NW 28)*	12,800	1/400 (1/32)	5,000	96·8	C_1 C_5 C_7	96·8 96·8 96·8	P B (BIN 1a) D T (BIN 1a) C_3 C_6	50 <50 " "			*For comments on these experiments see p. 63.* C_1, C_5, C_7 = Other infecting strains of type FG 4 used in experiment with serum. P B 99 (BIN 1a).
				Rep	12,800	1/800 (1/16)	40,000	93·75			P B (a) (BIN 1a) P B (b) (BIN 1a) P B (c) (BIN 1a) C_6	75 50 " <50			P B (BIN 1a) (a), (b) and (c) = different absorbing suspensions.
—	FG 27	P Ut (EAS 10) P PF (EAS 10) C_8 ? P Ut (WIN 33a) ? C_{10}	C_1 C_4 C_5 C_6 C_9	*FG 27 (SF 40)*	1,600	1/50 (1/32)	40,000	96·8	P Ut (EAS 10) P PF (EAS 10)	96·8 96·8	C_1 C_4 C_5 C_6 P Ut (WIN 33a)	50 <50 " "			C_8 = Another infecting strain of type FG 27. The infecting strains P Ut (WIN 33a) and C_{10} gave an indefinite reaction with the serum FG 27.
				Rep	800	1/50 (1/16)	40,000	93·75	C_8	93·75	C_9 C_{10}	<50 "			
—	FG 10	P_1 Ut (CUR 9)	C_1 C_2 P_5 PF (CUR 9) N_1 T (CUR 9)	*FG 10 (Dochez)*	6,400	1/200 (1/32)	10,000	96·8			P_1 Ut (CUR 9) P_5 PF (CUR 9) N_1 T (CUR 9) C_1 C_2	<50 " " " "			

TABLE II

A summary of the details relating to the absorption experiments shown in Table I

Character of the results of the absorption experiments (pp. 53–62).	*Sera.*	*Absorption experiments.*		*Samples of serum treated by strains other than the homologous strain.*		*Absorbing strains tested in these experiments.*				*Strains whose relationship to the homologous strain was not determined.*	
						Strains serologically identical with the homologous strain.		*Strains serologically different from the homologous strain.*			
		Total.	*Per Serum.*	*Total.*	*Per Serum.*	*Total.*	*Per Serum.*	*Total.*	*Per Serum.*	*Total.*	*Per Serum.*
A. Showing no irregularity or insignificant irregularities only. Group (a), p. 54.	64 + 3*	110	1·64	476	7·1	165	2·5	218	3·3	0	0
B. Showing various irregularities possibly attributable to defects of sera or absorbing strains. Group (b), p. 55.	7 + 3*	27	2·7	110	11·0	11	1·1	35	3·5	15	1·5
C. Unclassified Group (a) .. .. p. 55.	2	2	1	9	4·5	3	1·5	3	1·5	3	1·5
Total	76	139	1·8	595	7·8	179	2·4	256	3·4	18	0·2

* These 3 sera appear under A and B because certain experiments with them were satisfactory and other experiments unsatisfactory.

TABLE III

The titres of 76 sera prepared from strains of the patient-contact groups, and the character of the results of the absorption experiments performed with them

Comments on Table III

From the calculation of the average period of immunisation are excluded 9 sera :—

3 which were not tested until the 8th week ;

6 prepared by the administration of living cultures which inevitably prolonged the period of immunisation.

In certain experiments with the same agglutinating suspension, a 50 per cent. (1 tube) variation in the titre of the serum was recorded, and, rarely, a different agglutinating suspension gave an entirely different titre. To avoid unnecessary confusion these variations are not shown and the figure for each serum is the highest reached with any suspension. In the case of the sera of low titre, more than one suspension was almost invariably tested.

Samples of sera which were taken from 4 rabbits on different dates, with intervals of at least 6 weeks between the samplings, are counted as separate sera.

The character of the results of the absorption experiments.	*The titres of the sera.*									*Average period of immunisation (in weeks).*
	<1/800	*1/800*	*1/1,600*	*1/3,200*	*1/6,400*	*1/12,800*	*1/25,600*	*>1/25,600*	*Total.*	
Experiments showing no irregularities or insignificant irregularities. Group (a), p. 54.			12	16	12	11	8	5	64	(55 sera) 4·6
Experiments showing major degrees of absorption by heterologous strains suggestive of defects in the sera. Group (b), p. 55.	1	2	1*	1*					5	(5 sera) 10·0
Experiments showing various irregularities possibly attributable to defects in the absorbing strains or suspensions. Group (b), [pp. 55–59].			3‡					1†	4	(7 sera)
Unclassified experiments [p. 55]	1			1					2	
The serum P B 32 (NAS 18). Group b (iv), p. 57								1	1	6·5

* These titres were obtained by 2 courses of inoculations lasting 13 and 15 weeks respectively. At the end of the first period of inoculation the titres of both sera were 1/400.

‡ The sera which appear under A and B in Table II.

† The serum P Ut 44 (WOO 34) (p. 56).

TABLE IV

The serological relationships of the strains of 55 *patient-contact groups* (67 *patients*)

A summary of the results shown in Table I.

Note.—A brief resume of the epidemiological facts relating to each patient-contact group will be found on pp. 18-27.

Explanation of Table IV.

The strains of 38 patient-contact groups (48 patients) were tested by absorption experiments, the strains of 42 groups (52 patients) by direct (slide) agglutination; the strains of 25 of the above groups (33 patients) by both absorption experiments and direct (slide) agglutination. The Table is therefore subdivided:—

(i) The 25 patient-contact groups (33 patients) whose strains were tested both by absorption experiments and direct (slide) agglutination.

(ii) The 13 groups (15 patients) whose strains were tested by absorption experiments only:

(*a*) the type of the infecting strain of 2 patients was found, but the respiratory tract strains have not yet been tested with the type sera;

(*b*) the infecting strain of 13 patients did not react with any type serum on the slide.

(iii) The 17 groups (19 patients) whose strains were tested by direct (slide) agglutination only.

The abbreviations are those used in Table I. In addition:—

NS = swab not sent to this laboratory or not taken;
— = haemolytic streptococci not isolated from swab or swabs;
\+ = strains isolated but not tested serologically;
+TI = strains in a patient-contact group identical with each other;
+TD = strains in a group serologically different from each other;
+TNK = strains in a group whose serological relationship to each other was not determined by the experiments made.

In certain groups more than one case of puerperal fever occurred and a serum was prepared from the infecting strain of more than one patient. When the contacts of the one patient of the group were not the same persons as the contacts of the other patient, a horizontal line is drawn between the patients and contacts in question. When the contacts were shared by the patients the group is not so subdivided.

Thus:—

(1) *In the group* (*BRI 4*) there were two patients, P_1 and P_2, infected with identical strains. D_1 was a contact of P_1 but not of P_2; D_2 was a contact of P_2 but not P_1; the strain D_1T is identical with, but the strain D_2T is different from, the infecting strains. Two distinct colony types (P_2T_1 and P_2T_2) were isolated from the throat of P_2; the former was and the latter was not identical with the infecting strains.

(2) *In the group* (*SMI 24*) the two patients shared the contacts.

TABLE IV (*contd.*)

Strains isolated from members of the patient-contact groups.

The technique by which the strains of the groups were tested.	*Patient-contact group.*	*Infecting strains.* Uterus.	Blood.	Other source.	*Respiratory tract strains.* Patient. Nose.	Throat.	Contact. Nose.	Throat.	*Other strains.* Patient.	Contact.	*Notes.*
I. *Absorption experiments and direct (slide) agglutination.*	BOD 3	+TI			—	—	Ch_3 + Ch_1 — Ch_2 —	+TI +TI +TI	G + TD	Ch_1 Ear +TI	
	BRI 4	P_1 +TI			—	—	D_1 NS	+TI			
		P_2 +TI			+TD	P^{2}T$_1$ +TI P$_2$T$_2$+TD	D_2 NS	+TD			
	BUC 4a	P_1 +TI	+TI		—	+TI	N_1 +TI	—			H. suffered from tonsillitis 5 days after the confinement.
		P_2 +TI			—	+TI	D_1 NS N_2 +TI	— +TI			
		P_3 +TI			—	—	H +TI N_1 —	+TI —			
							N_2 —	+TI			
	BUS 5	+TI			NS	NS	Ch NS N_1 NS N_3 NS N_2 NS N_4 NS	+TI +TI +TD +TD +TD			
	CHA 7	+TI			+TI	—	Hw —	+TI	G + TD		
	CRO 8	+TI			—	—	N_1 — Hw —	+TI +TD			
	CUR 9	P_1 +TI	+TI		P$_1$ NS P$_2$ NS	+TI +TI	N_5 NS N_6 NS N_7 NS N_8 NS N_9 NS	+TI +TI +TD +TD +TD			In this group were four *puerperal* patients known to be infected with strains of the same serological type. The strains of only two patients, P_1 and P_4, and two contacts were fully investigated, however. The strain P_1 PF was isolated from a patient infected with a strain of the same type at the time of a clean operation in the *general surgical department* of the hospital in which the above-mentioned puerperal patients were confined. In the figures in the Tables, therefore, the group appears as having two patients and their contacts only.
		P_3 +TI									
		P_4 +TI P_6 +TNK			P$_4$ NS P$_6$ NS	NS +TNK	N_1 NS N_3 NS N_4 NS	+TI +TD +TNK			
	BUS 5	+TI			NS	NS	N_2 NS	+TI			
	CHA 7	+TI			+TI	—	Ch_1 NS N_1 NS N_3 NS N_2 NS N_4 NS	+TI +TI +TD +TD +TD	G + TD		
	CRO 8	+TI			—	—	N_1 — Hw —	+TI +TD			
	CUR 9	P_1 +TI	+TI		P$_1$ NS P$_2$ NS	+TI +TI	N_5 NS N_6 NS N_7 NS N_8 NS N_9 NS	+TI +TI +TD +TD +TD			In this group were four *puerperal* patients known to be infected with strains of the same serological type. The strains of only two patients, P_1 and P_4, and two contacts were fully investigated, however. The strain P_1 PF was isolated from a patient infected with a strain of the same type at the time of a clean operation in the *general surgical department* of the hospital in which the above-mentioned puerperal patients were confined. In the figures in the Tables, therefore, the group appears as having two patients and their contacts only.
		P_3 +TI									
		P_4 +TI P_6 +TNK			P$_4$ NS P$_6$ NS	NS +TNK	N_1 NS N_3 NS N_4 NS	+TI +TD +TNK			
	CUR 9	P_1 +TI	+TI		NS	NS	N_2 NS	+TI			
		P_3 +TI									
		P_4 +TI P_6 +TNK			P$_4$ NS P$_6$ NS	NS +TNK	N_1 NS N_3 NS N_4 NS	+TI +TD +TNK			
							N_2 NS	+TI			
	DUR 10	+TI			NS	NS	N_1 NS N_2 NS	+TI +TI			
	EDG 11	P_1 +TI			—	—	N_1 NS N_2 NS	+TI +TI			
		P_2 +TI			—	—	N_3 NS N_4 NS	+TI +TI			
		P_3 +TI			—	—	N_5 NS N_6 NS	+TI +TI			
		P_4 +TI			—	—	N_7 NS N_8 NS	+TI +TI			
		P_5 +TI			—	—	N_9 NS	+TI			
		P_6 +TI			—	—					
		P_7 +TI			—	—					
		P_8 +TI			—	—					
		P_9 +TI			—	—					
		P_{10} +TI			—	—					
		P_{11} +TI			—	—					
		P_{12} +TI			—	—					
		P_{13} +TI			—	—					
		P_{14} +TI			—	—					
		P_{15} +TI			—	—					
		P_{16} +TI			—	—					
		P_{17} +TI			—	—					
		P_{18} +TI			—	—					
		P_{19} +TI			—	—					
		P_{20} +TI			—	—					
		P_{21} +TI			—	—					
		P_{22} +TI			—	—					
		P_{23} +TI			—	—					
		P_{24} +TI			—	—					
		P_{25} +TI			—	—					
		P_{26} +TI			—	—					
		P_{27} +TI			—	—					
		P_{28} +TI			—	—					
		P_{29} +TI			—	—					
		P_{30} +TI			—	—					
		P_{31} +TI			—	—					
		P_{32} +TI			—	—					
		P_{33} +TI			—	—					

TABLE IV (*contd.*)

The technique by which the strains of the groups were tested.	Patient-contact group.	Strains isolated from members of the patient-contact groups.									Notes.
		Infecting strains.			Respiratory tract strains.				Other strains.		
					Patient.		Contact.				
		Uterus.	Blood.	Other source.	Nose.	Throat.	Nose.	Throat.	Patient.	Contact.	
I. *Absorption experiments and direct (slide) agglutination*—contd.	HAL 11	+TD			–	–	N –	+TD			
	HAR 12	P_1 +TI P_2 +TI			–	–	N_1 – N_2 –	+TI +TI			
				P_3 +TI (PI F)	P_3 –	+TI	–	–			
	HAY 13	+TI			+TI	–	N +TI	+TI			The nurse had a chronic infection of the antrum.
	LAW 14	+TI			+TI	–	N NS D NS	– –			
	LUF 16	+TI	+TI V +TI	PC +TI	+TI	–	N –	–			V = Thrombosed vein. PC = Pericardium.
	NAS 18		+TNK		+TNK	+TD	D NS N_1 – Hw NS N_2 –	+TNK +TNK +TNK +TD			
	OAK 19	P_1 +TI	+TI		–	–	D NS N_1 – N_2 –	+TI +TD +TD			The doctor was suffering from a quinsy at the time of the confinement.
		P_2 +TI			+TI	–	N_1 – N_2 –	+TD +TD			
	PAG 20	+TI	+TI		+TI	T_1 +TI T_2 +TD	D – N –	– –	G +TD		Patient's throat yielded 2 strains of different colonial appearance (P T_1 and P T_2).
	PIC 21		+TI		+TI	+TI	N NS	+TI			
	POP 22	+TI			+TI	+TI	D – N –	+TI +TI		Baby 1 PF +TI Baby 2 PF +TI	The two babies, who were in the same home as but not contacts of the patient, died of peritonitis.

	SAU 23	+TD			–	+TD	N NS	+TD	G +TD		
	SMI 24	P_1 +TI		P_1 PF +TI P_2 PF +TI	– NS	+TD NS	N_1 – N_2 – N_3 +TD N_4 –	+TI +TD – +TD			
	(D) TA 27		+TI		NS	–	D –	+TI			
	(R) TA 28	+TI			–	–	N –	+TI			
	TRI 30	+TI	+TI		+TI	+TI	N_1 +TI M – D + N_2 –	+TI +TI +TD +TI	G +TI		M = Maid.
	VEN 31a	+TI			–	–	D_1 NS D_2 NS N NS	+TI + +			A satisfactory serum has not yet been prepared from the strain P Ut (VEN); this infecting strain and the contact strain D_1 T, however, both effected complete absorption of the serum P Ut 98 (TEV 28a) prepared from a strain of the same type as the strain P Ut (VEN).
	WAR 32	+TI	+TI		NS	+TI	N_1 +TI N_2 +TD N_3 +TD N_4 +TD N_5 +TD N_6 +TD				The nose and throat swab of each contact were smeared together on the same plate. The strains are counted as throat strains.
	WOO 34	+TI			NS	+TI	D NS	+TNK	G +TI		
II. *Absorption experiments only.*	ARM 1	P_1 +TI P_2 +TI			– –	+TI +TI	N – N –	– –			
	BIN 1a	+TI	+TI		–	–	D_1 – D_2 –	+TD NS			
	BIR 2	+TD			–	–	N_1 – N_2 –	+TD +TD			
	BYR 6	+TNK			–	+TNK	NS	NS		H Ps+TNK	The husband was suffering from a septic hand at the time of the confinement.
	LEC 15	+TI		PF +TI	+TI	+	N – D –	+TI +			

TABLE IV (*contd.*)

The technique by which the strains of the groups were tested.	Patient-contact group.	Strains isolated from Members of the Patient-contact Groups.									Notes.
		Infecting strains.			Respiratory tract strains.				Other strains.		
					Patient.		Contact.				
		Uterus.	Blood.	Other source.	Nose.	Throat.	Nose.	Throat.	Patient.	Contact.	
II. *Absorption experiments only* —contd.	MAR 17	+TD			–	–	Ch_1 NS Ch_2 NS Hw NS	NS +TD +TD		Ch_1 Ear+TD	
	SEA 23a	+TNK			–	–	N_1 – N_2 –	+TNK +TNK			
	SOP 25	+TD			–	+TD	D NS	+TD			
	SPR 26	+TNK			NS	NS	NS	NS		ChPs+TNK	Pus from impetigo.
	TEV 28a	+TI			+TI	–	N –	+TI			
	TIP 29	+TI			–	+TI	NS	NS			
	VAN 31	P_1 +TI		P_2 Ps +TI	NS NS	NS NS	N NS	+TD			P_2 Ps = Pus from the wrist of patient P_2. The primary infecting strain of this patient was not sent.
	WHI 33	–	+TI		+TI	–	N NS	–			
III. *Direct (slide) agglutination only.*	AVE	+TI			NS	NS	M NS	+TI			M = The patient's mother, who had a sore throat before the confinement.
	BEV	P_1 +TI P_2 +TI			NS NS	NS NS	D_1 NS N_1 NS N_2 NS N_3 NS	+TD +TD +TD +TD			
	L BRO	+TI			NS	–	N – Ch+TI	+TD –			The child suffered from a cold a few days before the confinement.
	CROS	+TI			–	–	N – Hw +	+TI +TI			The handywoman had influenza just before the confinement.
	COO	+TI			–	–	N – H +TI	– +TI			H. developed tonsillitis 1 day before the confinement.
	CLA	+TD			–	–	N –	+TD			

	(M) ED	+TI			–	–	F – N –	+TI –			F = The patient's father, who had tonsillitis 1 month before the confinement.
	GUM	P_1 +TI			–	–	N_1 –	–			
		P_2 +TI			–	–	N_2 –	+TD			
	HIG	+TI			+TI	+TI	H – Ch_1 – Ch_2 – Ch_3 – Ch_4 –	+TD +TI +TI + +			Child 1 had suffered from otitis media shortly before the confinement.
	HOD	+TD	+		–	–	N_1 – N_2 – N_3 NS	+TD +TD NS			
	JON	+TI			NS	NS	Ch NS	+TI			Child developed scarlet fever 5 days after the confinement.
	MAS	+TI			–	–	N –	–		Ch F +TI	Child suffered from a septic finger for some days before the confinement.
	SMI F	+TI			NS	NS	Ch NS Hw NS	+TI +TI			
	SMI N	+TI			NS	NS	Ch_1 NS Ch_2 NS N NS Ch_3 NS Ch_4 NS	+TI +TI +TI + +			Child suffered from tonsillitis 1 month before the confinement.
	WIL	+TI			–	–	N –	+TD	Ps +TI		Pus from foot of the patient which was septic before the confinement.
	NEG	+TD			–	+TD	D – N –	+TD –			
	RIC	+TI			+TI	–	H NS D NS	+TI +TD		B_2 Brain +TI	The husband suffered from "influenza and sore throat" immediately before the confinement. The twin babies died of meningitis.

TABLE V

A summary of the results shown in Table IV: *the distribution of the respiratory tract and other strains found to be identical with the infecting strains among the 63 patients and their "groups of contacts" of 51 patient-contact groups.*

A comparison of the findings with those of Smith (1931 and 1933).

Note.—The significance of the terms "complete material" and "incomplete material" is discussed on p. 28.

	Incidence of Strains identical with the Infecting Strain.																		
	In the Respiratory Tract.															*In a septic focus.*			
	Patients.							*Contacts.*						*Patients and contacts.*					
		Nose.		*Throat.*				*Nose.*		*Throat.*									
Source of figures.	*Patients.*	*Swabs examined.*	*Strains identical with the infecting strain.*	*Swabs examined.*	*Strains identical with the infecting strain.*	*Patients yielding both a nose and a throat strain identical with the infecting strain.*	*Patients showing a possible source of infection.*	*Groups of swabs examined.*	*Strains identical with the infecting strain.*	*Groups of swabs examined.*	*Strains identical with the infecting strain.*	*Contacts yielding both a nose and a throat strain identical with the infecting strain.*	*Contacts showing a possible source of infection.*	*Possible source of infection in both patient and contact.*	*Possible source of infection in either patient or contact or both.*	*Patient.*	*Contact.*	*Patient or contact.*	*Total.*
This study.																			
Complete material ..	31	31	10 (32·3%)	31	9 (29%)	4	15 (48·4%)	31	5 (16·1%)	31	19 (61·3%)	4	20 (64·5%)	10	25 (80·7%)	1	1	2	27 (87%)
Incomplete material ..	32	16	4 (25%)	21	6 (28·6%)	1	9 (28%)	4	1 (25%)	27	15 (55·5%)	0	16 (50%)	4	21 (65·6%)	0	0	0	21 (65·6%)
All material ..	63	47	14 (29·8%)	52	15* (28·8%)	5	24 (38·1%)	35	6* (17·1%)	58	34 (58·6%)	4	36 (57·1%)	14	46 (73%)	1	1	2	48 (76·2%)
Smith.																			
1931 series	18	18	1 (5·5%)	18	0	0	1 (5·5%)	18	4 (22·2%)	18	9 (50%)	1	12 (66·6%)	0	13 (72·2%)	1	1	2	15 (83·3%)
1933 series	31	31	2 (6·5%)	31	7 (22·6%)	2	7 (22·6%)	31	5 (16·1%)	31	16 (51·6%)	2	19 (61·3%)	0	26 (84%)	0	0	0	26 (84%)
Both series	49	49	3 (6·1%)	49	7 (14·3%)	2	8 (16·3%)	49	9 (18·4%)	49	25 (51%)	3	31 (63·3%)	0	39 (79·6%)	1	1	2	41 (83·7%)

* One patient throat strain and the nose strain isolated from the group of swabs of the contacts of one patient were not examined serologically; in both cases a "possible source of infection" was found elsewhere.

CHARTS I and II

The results of direct agglutination in the water-bath

Reciprocal tests at a single dilution, namely, 1/32nd of the titre, of 36 sera and their homologous strains of various serological types.

Explanation of Charts I and II.

The sera and strains are designated as in Table I.
A blank space means that no test was performed.
— = no reaction obtained.
The circles indicate reactions of a serum with strains of its own serological type.
Five sera and strains were used in both tests.

Chart I

SERA		STRAINS	1 (ARM 1) P Ut	2 (BOD 3) P Ut	3 (BRI 4) P1 Ut	4 (BRI 4) P2 B	5 (BUS 5) P Ut	6 (CUR 9) P1 Ut	7 (CUR 9) P4 Ut	8 (CUR 9) P5 PF	9 (EAS 10) P Ut	10 (HAR 12) P Ut	11 (HAY 13) P Ut	12 (LUF 16) P Ut	13 (NAS 18) P B	14 (OAK 19) P Ut	15 (PAG 20) P B	16 (PIC 21) P B	17 (SOP 25) P Ut	18 (DTA 27) P B	19 (RTA 28) P Ut	20 (TRI 30) P Ut	21 (VAN 31) P Ut	22 (WAR 32) P B	23 (WHI 33) P B	24 (WIN 33a) P B	25 (WOO 34) P Ut	
P Ut 47 (ARM 1)	1		⊕	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
P Ut 70 (BOD 3)	2		–	⊕	–	+	–	+	+	±	–	–	⊕	–	–	–	–	±	–	–	⊕	–	–	–	–	+	2
P1 Ut 80 (BRI 4)	3		–	–	⊕	⊕	–	+	+	+	+	+	+	⊕	–	–	–	–	±	+	+	–	–	–	–	+	–	3
P2 B 64 (BRI 4)	4		±	–	tr ⊕	⊕	–	+	+	–	–	–	–	⊕	–	–	–	–	+	+	+	–	–		+	+	–	4
P Ut 21 (BUS 5)	5		–	–	–	+	⊕	+	+	+		–	–	–		–	±	⊕	+		±	–	+	–	–	+	–	5
P1 Ut 76 (CUR 9)	6		–	–	–	–	–	⊕	⊕	⊕	–	–	–	–	–	–	–	–	–	–	+	–	+		–	–	–	6
P4 Ut 77 (CUR 9)	7		–	–	–	–	–	⊕	⊕	tr	–	–	–	–	–	–	–	–	–	–	+	–	–				–	7
P5 PF 84 (CUR 9)	8		–	–	–	–	–	⊕	⊕	±	–	–	–	–	–	–	–	–	–	–	–	–	–				–	8
P Ut 58 (EAS 10)	9		–	–	–	–	–	+	+	–	⊕	–	–	–	–	–	–	+	±	±	–	–	–	–	–	–	–	9
P Ut 25 (HAR 12)	10		–	–	–	–	–	–	+	–	–	⊕	–	–	–	–	–	+	–			–	–	–	–	–	–	10
P Ut 52 (HAY 13)	11		–	±	–	–	–	–	–	–		–	⊕	–	–	–	–	–				⊕	–	–	–	–	–	11
P Ut 67 (LUF 16)	12		–	–	–	⊕		+	+	–	–	–	–	⊕	–	–	–	±	–	–	+	–	–				–	12
P B 32 (NAS 18)	13		–	–	–	–	–	–	–	–	–	–	–	–	⊕	–	–	–	–	–	–	–	–	–	–	–	–	13
P Ut 65 (OAK 19)	14		–	–	–	–	–	+	+	–	–	–	–	–	–	⊕	–	–	–	–	–	–	–	–	–	–	⊕	14
P B 62 (PAG 20)	15		–	–	–	–	–	–	–	–	–	–	–	–	–	–	⊕	–	–	–	–	–	–	–	–	–	–	15
P B 23 (PIC 21)	16		–	–	–	+	tr	+	+	–		–	–	–		–	–	⊕	–	–	–	–	–	–	–	–		16
P Ut 46 (SOP 25)	17		–	–		–	–	–	–			–	–	–	–	–	–	–	⊕	–	–	–	⊕	–		–		17
P B 22 (DTA 27)	18		–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	⊕	–	–	–	–	–	–	–	18
P Ut 71 (RTA 28)	19		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	⊕	–	–	–	–	–	–	19
P Ut 81 (TRI 30)	20		–	⊕	–	±	–	+	+	–	–	–	⊕	–	–	–	–	–	–	–	–	⊕	–	–	–	–	–	20
P Ut 31 (VAN 31)	21			–	–	–	±	+	+	–	–	+	–	–				–	–	+		–	–	⊕	–			21
P B 45 (WAR 32)	22		–		–	–	–	–	–			–	–	–	–	–	–	–	–	–	±	–	–	–	⊕	–		22
P B 35 (WHI 33)	23		–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	⊕	–	23
P B 97 (WIN 33a)	24			+	+	+	+	+	+								+					+	+			⊕	24
P Ut 44 (WOO 34)	25		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	⊕	–	–	–	–	–	–	–	–	–	25
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	

Chart II

SERA		STRAINS	1 (BIN 1a) P Ut	2 (BRI 4) P B	3 (BUC 4a) P1 Ut	4 (BUC 4a) P2 Ut	5 (CHA 7) P Ut	6 (CRO 8) P Ut	7 (CUR 9) P1 Ut	8 (LEC 15) P Ut	9 (NAS 18) P B	10 (POP 22) P Ut	11 (POP 22) B1 PF	12 (SMI 24) P1 Ut	13 (SMI 24) P2 PF	14 (DTA 27) P B	15 (WAR 32) P B	16 (WAR 32) N1 T	
P Ut 99 (BIN 1a)	1		⊕	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
P B 64 (BRI 4)	2		–	⊕	–	–	±	–	±	–	–	+	+	+	±	–	–	–	2
P1 Ut 102 (BUC 4a)	3		–	–	⊕	⊕	⊕	–	–	–	–	–	–	–	–	⊖	⊕	⊕	3
P2 Ut 101 (BUC 4a)	4		–	–	⊕	⊕	⊕	–	–	–	–	–	±	–	–	⊖	⊕	⊕	4
P Ut 92 (CHA 7)	5		–	–	⊕	±	⊕	–	–	–	–	–	–	–	–	⊖	⊕	±	5
P Ut 96 (CRO 8)	6		–	–	–	–	–	⊕	–	–	–	+	–	–	–	–	–	–	6
P1 Ut 76 (CUR 9)	7		–	–	–	–	–	–	⊕	–	–	+	–	–	–	–	–	–	7
P Ut 94 (LEC 15)	8		–	–	–	–	–	–	–	⊕	–	–	–	–	–	–	–	–	8
P B 32 (NAS 18)	9		–	–	–	–	–	–	–	–	⊕	–	–	–	–	–	–	–	9
P Ut 93 (POP 22)	10		–	–	–	–	–	–	–	–	–	⊕	⊕	–	–	–	–	–	10
B1 PF 95 (POP 22)	11		–	–	–	–	–	–	–	–	–	⊕	⊕	–	–	–	–	–	11
P1 Ut 90 (SMI 24)	12		–	–	–	–	–	–	–	–	–	–	–	⊕	tr	–	–	–	12
P2 PF 89 (SMI 24)	13		–	–	–	–	–	–	–	–	–	±	–	⊕	⊕	–	–	–	13
P B 22 (DTA 27)	14		–	–	⊕	⊕	⊕	–	–	–	–	–	–	–	–	⊖	⊕	⊕	14
P Ut 45 (WAR 32)	15		–	–	⊕	±	⊕	–	–	–	–	–	–	–	–	tr	⊕	±	15
N1 T 86 (WAR 32)	16		–	–	⊕	⊕	⊕	–	–	–	–	⊕	–	±	–	⊖	⊕	⊕	16
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	

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Enteric Infections:

No. 9. A Report upon the Use of Atropine as a Diagnostic Agent in Typhoid Infections. By H. F. Marris. [1917.] 1*s.* (1*s.* 1*d.*).

No. 48. A Report on the probable Proportion of Enteric Infections among Undiagnosed Febrile Cases invalided from the Western Front since October, 1916. By W. W. C. Topley, S. G. Platts, and C. C. Imrie. [1920.] 3*s.* (3*s.* 1½*d.*).

No. 179. Chronic Enteric Carriers and their Treatment. By C. H. Browning with others. [1933.] 1*s.* 6*d.* (1*s.* 7½*d.*).

See also BACTERIOLOGY; FOOD POISONING.

Epidemiology:

No. 75. The Schick Test, Diphtheria and Scarlet Fever. By S. F. Dudley. [1923.] 1*s.* (1*s.* 1½*d.*).

No. 111. The Spread of Droplet Infection in Semi-isolated Communities. By S. F. Dudley. [1926.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 120. An Inquiry into the Relationship between Housing Conditions and the Incidence and Fatality of Measles. By J. L. Halliday. [1928.] 1*s.* (1*s.* 1*d.*).

No. 137. Scarlet Fever, Diphtheria, and Enteric Fever, 1895–1914: A Clinical-Statistical Study. By E. W. Goodall, M. Greenwood, and W. T. Russell. [1929.] 2*s.* (2*s.* 1½*d.*).

No. 180. Epidemiological Study of Scarlet Fever in England and Wales since 1900. By Hilda M. Woods. [1933.] 1*s.* 3*d.* (1*s.* 4½*d.*).

No. 192. Housing Conditions and Respiratory Disease. Morbidity in a Poor-class Quarter and in a Rehousing Area in Glasgow. By C. M. Smith. [1934.] 9*d.* (10*d.*).

No. 195. Active Immunization against Diphtheria: its effect on the Distribution of Antitoxic Immunity and Case and Carrier Infection. By S. F. Dudley, P. M. May, and J. A. O'Flynn. [1934.] 3*s.* (3*s.* 2½*d.*).

See also SMALL-POX; TUBERCULOSIS; ETC.

Flying, Medical Problems of:

Reports of the Air Medical Investigation Committee:—

No. 28. The Sense of Balance and Stability in the Air. By Henry Head. [1919.] 9*d.* (10*d.*). (Included in No. 53.)

No. 37. The Effects of Diminished Tension of Oxygen, with especial reference to the Activity of the Adrenal Glands. By C. H. Kellaway. The Ear in relation to certain Disabilities in Flying. By S. Scott. [1919.] 1*s.* (1*s.* 1*d.*).

No. 53. The Medical Problems of Flying (including reports on oxygen want, selection of candidates for flying, sense of balance, and flying strain). [1920.] 6*s.* (6*s.* 4*d.*).

No. 84. The Application of the Air Force Physical Efficiency Tests to Men and Women. By L. D. Cripps. [1924.] 1*s.* 6*d.* (1*s.* 7½*d.*).

Food Poisoning:

No. 24. A Report on the Investigation of an Epidemic caused by *Bacillus aertrycke*. By H. Marrian Perry and H. L. Tidy. [1919.] 9*d.* (10*d.*).

No. 91. An Investigation of the Salmonella Group, with Special Reference to Food Poisoning. By W. G. Savage and P. Bruce White. [1925.] 3*s.* 6*d.* (3*s.* 8*d.*).

No. 92. Food Poisoning: a Study of 100 Recent Outbreaks. By W. G. Savage and P. Bruce White. [1925.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 103. Further Studies of the Salmonella Group. By P. Bruce White. [1926.] 5*s.* (5*s.* 2½*d.*).

Goitre:

No. 154. Iodine Supply and the Incidence of Endemic Goitre. By J. B. Orr. [1931.] 4*d.* (5*d.*). *See also* NUTRITION (Nos. 123, 201).

Haemoglobin: *see* BLOOD.

Hearing:

No. 166. Reports of the Committee upon the Physiology of Hearing. I.—The Localization of Sounds in the Median Plane. By J. H. Shaxby and F. H. Gage. II.—Some Factors in Auditory Localization. By H. E. O. James and Marion E. Massey. [1932.] 1*s.* (1*s.* 1½*d.*).

Heart:

No. 8. Report upon Soldiers returned as Cases of "Disordered Action of the Heart" (D.A.H.), or Valvular Disease of the Heart. By Sir Thomas Lewis. [1917.] 1*s.* (1*s.* 1*d.*).

No. 147. The Electrocardiogram. By W. H. Craib. [1930.] 1*s.* 3*d.* (1*s.* 4½*d.*).

Heredity: *see* IMMUNITY (No. 196).

Immunity:

No. 194. The Chemistry of Antigens and Antibodies. By J. R. Marrack. [1934.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 196. The Inheritance of Resistance to Infection in Animal Species. A Review of the Published Experimental Data. By A. Bradford Hill. [1934.] 1*s.* 3*d.* (1*s.* 4½*d.*).

See also EPIDEMIOLOGY.

Industrial Health:

The Annual Reports of the Industrial Health (formerly Fatigue) Research Board, and special reports on particular subjects, are published for the Council in separate series. The subjects dealt with include accident causation, rest pauses, spells of work, movement study, vocational selection, and problems of particular industries. A list can be supplied on application to the Secretary of the Board, 38, Old Queen Street, Westminster, S.W.1.

Influenza :

No. 36. Studies of Influenza in Hospitals of the British Armies in France, 1918. [1919.] 3*s.* 6*d.* (3*s.* 8*d.*).

No. 63. Studies in the Aetiology of Epidemic Influenza. By J. McIntosh. [1922.] 2*s.* 6*d.* (2*s.* 7*d.*).

Jaundice :

No. 113. Spirochaetal Jaundice. By G. Buchanan. [1927.] 4*s.* (4*s.* 2*d.*).

Light Treatment :

No. 131. Irradiation and Health. Two Experimental Studies. By Dora Colebrook. [1929.] *Out of print.*

Malaria : *see* QUININE.

Maternal Mortality : *see* CHILD LIFE and STREPTOCOCCAL INFECTIONS.

Measles : *see* EPIDEMIOLOGY (No. 120).

Milk :

No. 206. The Bacteriological Grading of Milk. By G. S. Wilson. [1935.] *See also* TUBERCULOSIS, No. 189.

Miners' Dietaries : *see* NUTRITION.

Miners' Diseases, etc. :

No. 89. Report on Miners' "Beat Knee," "Beat Hand," and "Beat Elbow." By E. L. Collis and T. L. Llewellyn. [1924.] 1*s.* 6*d.* (1*s.* 7*d.*). *See also* JAUNDICE (No. 113).

Miners' Nystagmus : *see* VISION.

Nephritis :

No. 43. Albuminuria and War Nephritis among British Troops in France. By H. MacLean. [1919.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 142. A Classification of Bright's Disease. By Dorothy S. Russell. [1929.] 8*s.* 6*d.* (8*s.* 10*d.*).

No. 178. A Study of Nephritis and Allied Lesions. By J. Gray. [1933.] 2*s.* 6*d.* (2*s.* 8½*d.*).

Nerve Injuries :

Reports of the Committee upon Injuries of the Nervous System :—

No. 54. The Diagnosis and Treatment of Peripheral Nerve Injuries. [1920.] 2*s.* (2*s.* 1½*d.*).

No. 88. Injuries of the Spinal Cord and Cauda Equina. [1924.] 1*s.* 6*d.* (1*s.* 7½*d.*).

Nutrition :

No. 13. An Enquiry into the Composition of Dietaries, with special reference to the Dietaries of Munition Workers. By Viscount Dunluce and Major Greenwood. [1918.] *Out of print.*

No. 87. Report on the Nutrition of Miners and their Families. By the Committee upon Quantitative Problems in Human Nutrition. [1924.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 105. Diets for Boys during the School Age. By H. C. Corry Mann. [1926.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 123. Iodine in Nutrition : a Review of Existing Information. By J. B. Orr and I. Leitch. [1929.] *See also* GOITRE (No. 154). *Out of print.*

No. 135. The Carbohydrate Content of Foods. By R. A. McCance and R. D. Lawrence. [1929.] *Out of print.*

No. 146. The Antiscurvy Vitamin in Apples. By Mary F. Bracewell, E. Hoyle, and S. S. Zilva. [1930.] 9*d.* (10*d.*).

No. 151. A Study in Nutrition. An Inquiry into the Diet of 154 Families of St. Andrews. By E. P. Cathcart and A. M. T. Murray, assisted by M. Shanks. *Out of print.*

No. 155. Studies of Nutrition : The Physique and Health of Two African Tribes. By J. B. Orr and J. L. Gilks. [1931.] 2*s.* (2*s.* 2*d.*).

No. 158. The Quantitative Estimation of Vitamin D by Radiography. By R. B. Bourdillon, H. M. Bruce, C. Fischmann, and T. A. Webster. [1931.] 1*s.* (1*s.* 1½*d.*).

No. 165. Studies in Nutrition. An Inquiry into the Diet of Families in Cardiff and Reading. By E. P. Cathcart and A. M. T. Murray, assisted by M. Shanks. [1932.] 6*d.* (7*d.*).

No. 167. Vitamins : A Survey of Present Knowledge. By a Committee appointed jointly by the Lister Institute and Medical Research Council [1932.] 6*s.* 6*d.* (7*s.* 0½*d.*).

No. 175. Vitamin Content of Australian, New Zealand, and English Butters. By M. E. F. Crawford, E. O. V. Perry, and S. S. Zilva. [1932.] 1*s.* (1*s.* 1*d.*).

No. 187. The Chemistry of Flesh Foods and their Losses on Cooking. By R. A. McCance and H. L. Shipp. [1933.] 2*s.* 6*d.* (2*s.* 8½*d.*).

No. 201. The Determination of Iodine in Biological Substances. By C. O. Harvey. [1935.] 1*s.* (1*s.* 1½*d.*).

See also CHILD LIFE ; RICKETS ; DENTAL DISEASE ; STANDARDS (No. 202).

Pituitary Extract : *see* STANDARDS.

Pneumonia :

No. 79. Bacteriological and Clinical Observations on Pneumonia and Empyemata, with special reference to the Pneumococcus and to Serum Treatment. By E. E. Glynn and Lettice Digby. [1923.] 5*s.* (5*s.* 3*d.*).

Pneumothorax, Artificial : *see* TUBERCULOSIS.

Print, Legibility of : *see* VISION.

Protozoan Infections :

No. 59. A Report on the Occurrence of Intestinal Protozoa in the inhabitants of Britain. By Clifford Dobell. [1921.] 2*s.* (2*s.* 1½*d.*).

Psychology :

No. 170. Studies in the Psychology of Delinquency. By G. W. Pailthorpe. [1932.] 2*s.* (2*s.* 2*d.*).

Quinine :

No. 96. Clinical Comparisons of Quinine and Quinidine. By the Committee upon Cinchona Derivatives and Malaria. [1925.] 1*s.* (1*s.* 1*d.*).

Radium :

No. 62. Medical Uses of Radium: Studies of the Effects of Gamma Rays from a large Quantity of Radium. By various authors. [1922.] 5*s.* (5*s.* 3*d.*).

No. 90. Medical Uses of Radium : Summary of Reports from Research Centres for 1923. [1924.] 1*s.* (1*s.* 1*d.*).

No. 102. Ditto for 1924. [1926.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 112. Ditto for 1925. [1926.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 116. Ditto for 1926. [1927.] 1*s.* (1*s.* 1½*d.*).

No. 126. Ditto for 1927. [1928.] 1*s.* (1*s.* 1½*d.*).

No. 144. Ditto for 1928. [1929.] 1*s.* (1*s.* 1*d.*).

No. 150. Ditto for 1929. [1930.] 9*d.* (10*d.*).

No. 160. Ditto for 1930. [1931.] 1*s.* (1*s.* 1*d.*).

No. 174. Ditto for 1931. [1932.] 1*s.* 3*d.* (1*s.* 4½*d.*).

No. 186. Ditto for 1932. [1933.] 1*s.* (1*s.* 1*d.*).

No. 197. Ditto for 1933. [1934.] 9*d.* (10*d.*).

No. 204. Ditto for 1934. [1935.] 1*s.* (1*s.* 1½*d.*).

Respiration :

No. 198. Test for Respiratory Efficiency. By A. Moncrieff. [1934.] 1*s.* (1*s.* 1½*d.*).

Rheumatism : *see* CHILD LIFE (No. 114).

Rickets :

No. 20. A Study of Social and Economic Factors in the Causation of Rickets, with an Introductory Historical Survey. By L. Findlay and Margaret Ferguson. [1918.] *Out of print.*

No. 61. Experimental Rickets. By E. Mellanby. [1921.] 4*s.* (4*s.* 2*d.*).

No. 68. Rickets : the Relative Importance of Environment and Diet as Factors in Causation. By H. Corry Mann. [1922.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 71. The Aetiology and Pathology of Rickets from an experimental point of view. By V. Korenchevsky. [1922.] 4*s.* (4*s.* 3*d.*).

No. 77. Studies of Rickets in Vienna, 1919–22. [1923.] 7*s.* 6*d.* (7*s.* 10½*d.*).

No. 93. Experimental Rickets : The Effect of Cereals and their Interaction with other factors of Diet and Environment in producing Rickets. By E. Mellanby. [1925.] 3*s.* 6*d.* (3*s.* 8*d.*).

Salvarsan: *see* VENEREAL DISEASES; STREPTOCOCCAL INFECTIONS; STANDARDS, BIOLOGICAL (No. 128).

Scarlet Fever : *see* EPIDEMIOLOGY (Nos. 137, 180).

Scurvy : *see* NUTRITION (No. 146).

Shock, Surgical :

Reports of the Committee on Surgical Shock and Allied Conditions :—

No. 25. Wound-Shock and Haemorrhage. [1919.] 4*s.* (4*s.* 5½*d.*).

No. 26. Traumatic Toxaemia as a Factor in Shock. [1919.] 1*s.* (1*s.* 1*d.*).

No. 27. Blood Volume Changes in Wound-Shock and Primary Haemorrhage. By N. M. Keith. [1919.] 9*d.* (10*d.*).

Small-pox :

No. 98. Studies of the Viruses of Vaccinia and Variola. By M. H. Gordon. [1925.] 3*s.* 6*d.* (3*s.* 8½*d.*).

No. 106. Small-pox and Climate in India. Forecasting of Epidemics. By Sir Leonard Rogers. [1926.] 2*s.* (2*s.* 1½*d.*).

No. 143. Diagnostic Value of the "Vaccinia Variola" Flocculation Test. By W. L. Burgess, James Craigie, and W. J. Tulloch. [1929.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 156. Further Investigations on the Variola-Vaccinia Flocculation Reaction. By James Craigie and W. J. Tulloch. [1931.] 3*s.* (3*s.* 2½*d.*).

Spectroscopy :

No. 177. Apparatus for the Rapid Study of Ultra-Violet Absorption Spectra. By J. St. L. Philpot and E. H. J. Schuster. [1933.] 1*s.* 3*d.* (1*s.* 4½*d.*).

Spinal Deformities : *see* SURGERY (No. 161).

Standards, Biological :

No. 69. I.—Pituitary Extracts. By J. H. Burn and H. H. Dale. [1922.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 128. II.—Toxicity Tests for Novarsenobenzene (Neosalvarsan). By F. M. Durham, J. H. Gaddum, and J. E. Marchal. [1929.] 1*s.* 9*d.* (1*s.* 10*d.*).

No. 183. III.—Methods of Biological Assay depending on a Quantal Response. By J. H. Gaddum. [1933.] 1*s.* (1*s.* 1*d.*).

No. 202. IV.—The Standardisation and Estimation of Vitamin A. Edited by E. M. Hume and H. Chick. [1935.]

See also VENEREAL DISEASES (No. 44) and NUTRITION (No. 158).

Statistics (MISCELLANEOUS).

No. 16. A Report on the Causes of Wastage of Labour in Munition Factories. By Major Greenwood. [1918.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 60. The Use of Death-rates as a Measure of Hygienic Conditions. By John Brownlee. [1922.] 3*s.* (3*s.* 1½*d.*).

No. 95. Internal Migration and its Effects upon the Death-rates : with Special Reference to the County of Essex. By A. B. Hill. [1925.] 3*s.* 6*d.* (3*s.* 8*d.*).

Streptococcal Infections :

No. 119. A Study of some Organic Arsenical Compounds with a view to their use in certain Streptococcal Infections. By L. Colebrook. [1928.] 1*s.* 3*d.* (1*s.* 4*d.*)

No. 205. The Source of Infection in Puerperal Fever due to Haemolytic Streptococci. By Dora C. Colebrook. [1935.]

Surgery :

No. 125. A Study of Intracranial Surgery. By H. Cairns. [1929.] *Out of print.*

No. 138. The Preparation of Catgut for Surgical Use. By W. Bulloch, L. H. Lampitt, and J. H. Bushill. [1929.] 4*s.* (4*s.* 3*d.*).

No. 161. The Intervertebral Discs. Observations on their Normal and Morbid Anatomy in relation to certain Spinal Deformities. By O. A. Beadle. [1931.] 2*s.* (2*s.* 2*d.*).

See also BURNS ; SHOCK, SURGICAL.

T.N.T. Poisoning :

No. 11. The Causation and Prevention of Tri-nitro-toluene (T.N.T.) Poisoning. By Benjamin Moore. [1917.] 1*s.* (1*s.* 1½*d.*).

No. 58. T.N.T. Poisoning and the Fate of T.N.T. in the Animal Body. By W. J. O'Donovan and others. [1921.] 3*s.* (3*s.* 1½*d.*).

Tuberculosis :

No. 1. First Report of the Special Investigation Committee upon the Incidence of Phthisis in relation to Occupations.—The Boot and Shoe Trade. [1915.] 3*d.* (3½*d.*).

No. 18. An Investigation into the Epidemiology of Phthisis Pulmonalis in Great Britain and Ireland. Parts I and II. By John Brownlee. [1918.] *Out of print.*

No. 22. An Inquiry into the Prevalence and Aetiology of Tuberculosis among Industrial Workers, with special reference to Female Munition Workers. By Major Greenwood and A. E. Tebb. [1919.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 33. Pulmonary Tuberculosis : Mortality after Sanatorium Treatment. By Noel D. Bardswell and J. H. R. Thompson. [1919.] 2*s.* (2*s.* 2*d.*).

No. 46. An Investigation into the Epidemiology of Phthisis in Great Britain and Ireland : Part III. By John Brownlee. [1920.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 67. Report on Artificial Pneumothorax. By L. S. T. Burrell and A. S. MacNalty. [1922.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 76. Tuberculosis in Insured Persons accepted for Treatment by the City of Bradford Health Committee. By H. Vallow. [1923.] 6*d.* (7*d.*).

No. 83. Tuberculosis of the Larynx. By Sir St. Clair Thomson. [1924.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 85. An Inquiry into the After-Histories of Patients treated at the Brompton Hospital Sanatorium at Frimley during the years 1905–14. By Sir P. H.-S. Hartley, R. C. Wingfield, and J. H. R. Thompson. [1924.] 1*s.* 6*d.* (1*s.* 7*d.*).

No. 94. Tuberculin Tests in Cattle, with special reference to the Intradermal Test. By the Tuberculin Committee. [1925.] 3*s.* (3*s.* 3*d.*).

No. 122. The Intradermal Tuberculin Test in Cattle : Collected Results of Experience. By J. B. Buxton and A. S. MacNalty. [1928.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 149. Tuberculosis in Man and Lower Animals. By H. H. Scott. [1930.] *Out of print.*

No. 152. Studies of Protection against Tuberculosis : Results with B. C. G. Vaccine in Monkeys. By A. Stanley Griffith. [1931.] 9*d.* (10½*d.*).

No. 164. The Value of Tuberculin Tests in Man with special reference to the Intracutaneous Test. By P. D'Arcy Hart. [1932.] 2*s.* (2*s.* 6*d.*).

No. 172. Tuberculosis Disease in Children : Its Pathology and Bacteriology. By J. W. S. Blacklock. [1932.] 3*s.* (3*s.* 6*d.*).

No. 182. Tuberculous Bacillaemia, A Review. By G. S. Wilson. [1933.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 184. The Eradication of Bovine Tuberculosis. By L. Jordan. [1933.] 2*s.* (2*s.* 2*d.*).

No. 189. Tuberculous Infection in Milk. A Report by the Department of Health for Scotland. [1933.] 9*d.* (10*d.*).

Venereal Diseases :

No. 14. The Wassermann Test. By the Committee upon Pathological Methods. [1918.] *Out of print : see No. 129.*

No. 19. The Laboratory Diagnosis of Gonococcal Infections. Methods for the Detection of *Spironema pallidum*. By the Bacteriological Committee. *New Edition.* [1923.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 21. The Diagnostic Value of the Wassermann Test. By the Committee upon Pathological Methods. [1918.] *Out of print.*

No. 23. An Analysis of the Results of Wassermann Reactions in 1,435 Cases of Syphilis or Suspected Syphilis. By Paul Fildes and R. J. G. Parnell. [1919.] 2*s.* (2*s.* 1*d.*).

No. 41. (I.) An Investigation into the Ultimate Results of the Treatment of Syphilis with Arsenical Compounds. By Paul Fildes and R. J. G. Parnell. (II.) A Clinical Study of the Toxic Reactions which follow the Intravenous Administration of "914". By R. J. G. Parnell and Paul Fildes. [1919.] 2*s.* (2*s.* 1*d.*).

No. 44. Reports of the Special Committee upon the Manufacture, Biological Testing, and Clinical Administration of Salvarsan and of its Substitutes. I. [1919.] 1*s.* (1*s.* 1*d.*).

No. 45. Unsuspected Involvement of the Central Nervous System in Syphilis. By Paul Fildes, R. J. G. Parnell, and H. B. Maitland. [1920.] 1*s.* (1*s.* 1*d.*).

No. 47. The Accuracy of Wassermann Tests, applied before and after death, estimated by Necropsies. I. The Wassermann Test applied before death. By H. M. Turnbull. [1920.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 55. (I.) Results of the Examination of Tissues from Eight Cases of Death following Injections of Salvarsan. By H. M. Turnbull. (II.) The Influence of Salvarsan Treatment on the Development and Persistence of Immunity, as indicated by Measurements of Agglutinins. By E. W. Ainley Walker. [1920.] 3*s.* (3*s.* 1½*d.*).

No. 66. Toxic Effects following the Employment of Arsenobenzol Preparations. By the Salvarsan Committee. [1922.] 2*s.* (2*s.* 1½*d.*).

No. 78. The Serum Diagnosis of Syphilis : The Wassermann and Sigma Reactions compared. [1923.] 5*s.* 6*d.* (5*s.* 9*d.*).

No. 107. The Effect of Treatment on the Wassermann Reactions of Syphilitic Patients. By E. E. Glynn, R. E. Roberts, and P. M. Bigland. [1926.] 3*s.* 6*d.* (3*s.* 8*d.*).

No. 129. The Wassermann Test. Technical Details of No. 1 Method M.R.C. (Modified). By E. J. Wyler. [1929.] 9*d.* (10*d.*).

No. 132. The Treatment of Syphilis : A Survey of Records from St. Thomas's Hospital. By L. W. Harrison. [1929.] 2*s.* (2*s.* 1½*d.*).

Ventilation, etc. :

No. 32. The Science of Ventilation and Open-air Treatment. Part I. By Leonard Hill. [1919.] 10*s.* (10*s.* 5½*d.*).

No. 52. The Science of Ventilation and Open-air Treatment. Part II. By Leonard Hill. [1920.] 6*s.* (6*s.* 4½*d.*).

No. 73. The Kata-thermometer in Studies of Body Heat and Efficiency. By Leonard Hill and others. [1923.] 5*s.* (5*s.* 2½*d.*).

No. 100. Methods of Investigating Ventilation and its Effects. By H. M. Vernon and others. [1926.] 2*s.* (2*s.* 1½*d.*).

No. 199. Physical Methods for the Estimation of the Dust Hazard in Industry. By H. L. Green and H. H. Watson. [1935.] 1*s.* (1*s.* 1½*d.*).

Vision :

No. 65. First Report of the Miners' Nystagmus Committee. [1922.] *Out of print.*

No. 80. Second Report of the Miners' Nystagmus Committee. [1923.] 9*d.* (10*d.*).

No. 110. The Legibility of Print. By R. L. Pyke. [1926.] 4*s.* (4*s.* 2*d.*).

No. 176. Third Report of the Miners' Nystagmus Committee. [1932.] 9*d.* (10*d.*).

Reports of the Committee on the Physiology of Vision :

No. 104. I. Illumination and Visual Capacities. By R. J. Lythgoe. [1926.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 127. II. Dark Adaptation (a Review of the Literature). By Dorothy Adams. [1929.] 5*s.* (5*s.* 2½*d.*).

No. 130. III. Two Studies in the Psychology of Reading. By M. D. Vernon and R. W. Pickford. [1929.] 2*s.* (2*s.* 1½*d.*).

No. 133. IV. Experiments on Binocular Vision. By N. M. S. Langlands. [1929.] 2*s.* 6*d.* (2*s.* 8*d.*).

No. 134. V. The Adaptation of the Eye : its Relation to the Critical Frequency of Flicker. By R. J. Lythgoe and K. Tansley. [1929.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 136. VI. Some Experiments on Peripheral Vision. By Myer Salaman. [1929.] 2*s.* 6*d.* (2*s.* 7*d.*).

No. 139. VII. A Re-determination of the Trichromatic Mixture Data. By W. D. Wright. [1929.] 1*s.* 3*d.* (1*s.* 4*d.*).

No. 148. VIII. The Movements of the Eyes in Reading. By M. D. Vernon. [1930.] 9*d.* (10*d.*).

No. 163. IX. Psychological Factors in Peripheral Vision. By G. C. Grindley. [1931.] 1*s.* (1*s.* 1*d.*).

No. 173. X. The Measurement of Visual Acuity. By R. J. Lythgoe, [1932.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 181. XI. Individual Differences in Normal Colour Vision. By W. O'D. Pierce. [1933.] 2*s.* (2*s.* 1½*d.*).

No. 185. XII. Colour Vision Requirements in the Royal Navy. [1933.] 1*s.* (1*s.* 1½*d.*).

No. 188. XIII. Determination of the Sensitiveness of the Eye to Differences in the Saturation of Colours. By L. C. Martin, F. L. Warburton, and W. J. Morgan. [1933.] 1*s.* (1*s.* 1*d.*).

No. 200. XIV. Characteristics of Dichromatic Vision. By F. H. G. Pitt. [1935.] 1*s.* 3*d.* (1*s.* 4½*d.*).

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by

G. S. WILSON

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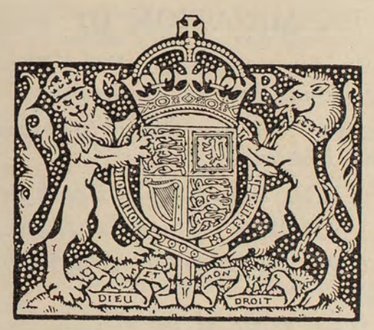

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TABLE VI

The incidence of the types described by Griffith in a series of puerperal infecting strains: the results of direct (slide) agglutination tests.

The distribution among 18 Griffith type strains of the 85 infecting strains which showed a definite reaction.

The Griffith type strains are designated as in Table I.

Tests of 121 puerperal injecting strains (derived from 121 patient-contact groups).								*Strains not tested on account of failure to secure a usable suspension.*
Griffith types found among puerperal strains		*Puerperal strains identical with these types.*	*Total puerperal strains.*	*Percentage*		*Strains not giving conclusive results.*	*Strains not reacting with any serum.*	
The Griffith types represented.	*Griffith type strains.*			*of all strains typed (85).*	*of all strains tested (121).*			
More than five times (5).	1 25 2 5 14	14 9 8 8 7	46	54·1	38·0			
More than four times (7).	3 11	5 5	56	65·9	46·3			
More than three times (11).	13 6 9 17	4 4 4 4	72	84·7	59·5			
Three times or less (18).	19 4 27 8 12 22 15	3 3 2 2 1 1 1	85	100	70·2	11*	25*	4

* It is probable that, in the case of some, at least, of these strains, the failure to react with a serum was due to insensitivity of the suspension. For this reason the percentages are calculated as shown in columns (5) and (6).