# A STUDY OF THE MECHANISMS OF TRANSMISSION OF RESPIRATORY-TRACT PATHOGENIC BACTERIA.

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PART 1:- INTRODUCTION AND REVIEW.

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CONTENTS OF PART 1b:

THE TRANSMISSION OF RESPIRATORY-TRACT TUBERCULOSIS



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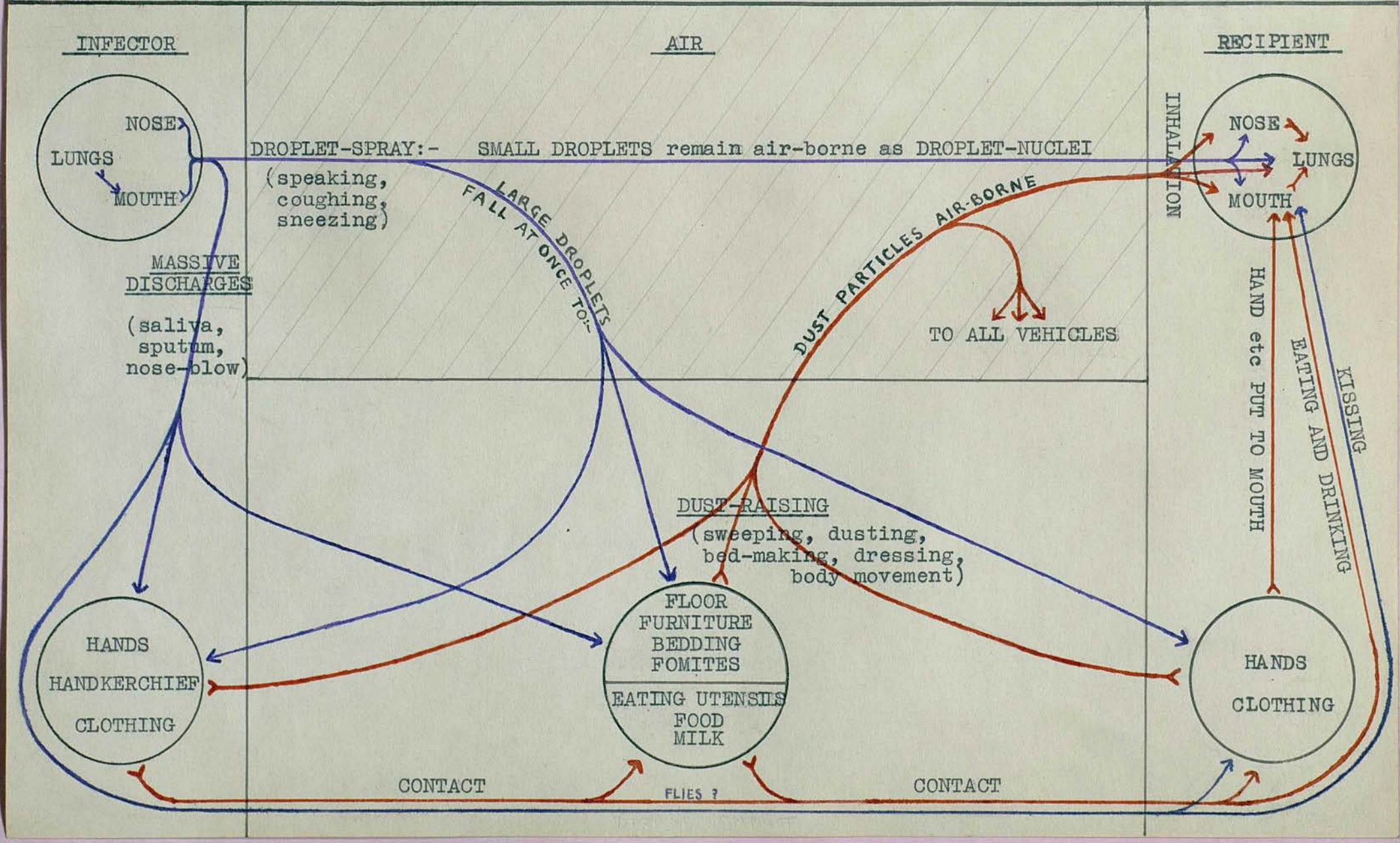
FIGURE I :- POSSIBLE MECHANISMS OF TRANSMISSION OF RESPIRATORY-TRACT PATHOGENIC ORGANISMS.



(1) General Introduction.

Infection with respiratory-tract pathogenic microorganisms is responsible for a large proportion of all sickness and death in civilised communities in temperate climates. Prevention of such infection remains an outstanding problem, probably the most urgent and serious confronting medical science. It seems that unless positive measures of prevention are undertaken, the incidence of respiratory infections will not lessen spontaneously. With the improved sanitation and the better living conditions of modern town and city life, many infectious diseases which were common in the past have now become infrequent; this has been the case with cholera, plague and typhoid fever. However, comparable improvement is not apparent in the case of the respiratory infections. Indeed, the structure of present-day urban society seems to be suited ideally to the spread of these infections; transmission of the pathogenic organisms undoubtedly is facilitated by the frequent sharing of communal enclosed air-spaces by large and changing populations, as in buses, shops, factories, offices, schools, cinemas and tenements.

Development of methods for preventing the spread of respiratory infections is rendered difficult because the manner of spread is not yet fully understood. A number of different mechanisms of transmission appear possible/

possible, and the relative importance of each of these is unknown. Infection may be transmitted by contact, by fomites, by eating utensils, by food or milk, by direct spraying with projectile secretion-droplets, by airborne droplet-nuclei, or by airborne dust-particles. Extant evidence makes it clear that each of these mechanisms is physically and biologically possible. What is not yet known is the frequency with which each mechanism causes infection under normal conditions. This can not be discovered by consideration of a few classic investigations or "crucial experiments", but only by accumulation of numerous observations from many different sources covering the wide range of conditions naturally existent in the community.

For this reason there has been included in the present thesis an extensive review of the observations which have been published by previous investigators (Part 1). The information relating to the transmission of pulmonary tuberculosis has been reviewed in a separate section (Part 1b) from that relating to the transmission of the other infections, which mainly are of the upper respiratory tract (Part 1a).

In addition to this review, an account is given (in Parts 2-6) of the author's experimental studies on various aspects of the problem of transmission of the respiratory infections; these relate particularly to the mechanisms of infection by droplet-spray and by dust,/

dust, to the natural environmental distribution of certain pathogenic bacteria by infected persons, to the extent of naturally-occurring contamination of the air with pathogenic bacteria, and to methods for bacteriological examination of the air of dwelling places.

(2) Incidence and Importance of Respiratory Infections.

That a major proportion of sickness and death is due to respiratory infection has been emphasised by Wells and Wells (1936) and by Mudd (1944) with reference to the morbidity and mortality figures published by the public health service of the U.S.A..

The reports of the Registrar-General (1932-1944; 1944) show that in Scotland, England and Wales during the ten years from 1931 to 1940 there was a total of 5,642,240 deaths from all causes. Of these deaths, 1,218,843, or 22%, were due to the respiratory infections, to bronchitis, pneumonia (including pleurisy and empyema), respiratory-tract tuberculosis, influenza, diphtheria, whooping cough, scarlet fever, septic sore throat, otitis media, mastoiditis, measles, varicella, rubella and small-pox. Not all cases in these disease categories can be regarded as the direct consequence of exogenous infection, of the acquisition of pathogenic microorganisms from other persons. A large proportion of the 369,476 fatal cases of bronchitis and a fair proportion of the 351,487 fatal cases of pneumonia probably were due primarily to heart failure, nephritis, allergy, exposure to noxious vapours, anaesthesia or nutritional deficiency, with consequent impairment of the defence mechanisms of the lower respiratory tract and endogenous infection with commensal bacteria from the throat. On the other hand,/

hand, deaths in certain further categories must be added to the score attributable to the acquisition of respiratory-tract microorganisms from other persons: namely, most of the 64,808 deaths from meningitis, erysipelas, puerperal infection and other non-respiratory infections with bacteria of respiratory-tract origin, about half of the 57,776 deaths from non-respiratory tuberculosis (i.e. those due to the human-type bacillus), and, if it is assumed that rheumatic fever is the result of streptococcal infection of the throat, a big proportion of the 277,181 deaths from rheumatic fever, pericarditis, rheumatic myocarditis, acute endocarditis and chronic valvular disease of the heart. It seems justifiable to conclude that infection with the respiratory-tract pathogenic microorganisms was responsible for about a fifth of all deaths occurring in Britain during the years from 1931 to 1940.

The serious feature of this mortality from respiratory infections is that it bears especially heavily on young persons; it thus differs from the mortality due to "senility diseases" such as cancer, which for the most part anticipates inevitable death by a short time only and so does not cause any great loss to the community. Figures given in the reports of the Registrar-General (1932-44) show that in England and Wales during the ten years from 1931 to 1940 infection with respiratory-tract pathogenic microorganisms/

microorganisms was responsible for about half of all deaths among young adults of 15 to 30 years of age.

The respiratory infections are foremost among the causes of incapacitating sickness. Because of their common occurrence, the minor respiratory infections such as the common cold, pharyngitis, bronchitis and influenza are of particular importance in this respect. Figures given in the reports of the Department of Health for Scotland (1936, 1937, 1939) show that during the three years from 1934 to 1937 there were 64,997,542 days of incapacity from all causes among the insured population of Scotland (about 1.7 million). Of these, 14,236,090, or 22%, were due to bronchitis, influenza, respiratory-tract tuberculosis, pneumonia, tonsillitis, pharyngitis, laryngitis, diphtheria, scarlet fever, and diseases of nose, sinuses, ear and mastoid. Making allowance for the omission of some of these cases as being endogenous infections, and the addition of other cases in which respiratory-tract bacteria infected non-respiratory parts of the body, it seems justifiable to conclude that about a fifth of all sickness and incapacity was due to infection with respiratory-tract pathogenic microorganisms.

Figures given by Slater (1946) for 2870 persons interviewed at random in Britain during January, show that 53% of all incapacity was due to the common cold, influenza, bronchitis, pharyngitis, tonsillitis, and diseases/

diseases of ear and mastoid. Collins (1940) gave comparable figures for 39,185 persons in America who were interviewed periodically for one year; out of a total of 1,013,501 days of incapacity from all causes, 19% were attributable to "respiratory disease" (half to the common cold, pharyngitis, tonsillitis, laryngitis, bronchitis and influenza). Gafafer (1943) reported the number of days absence from work due to different causes among the employees of an American public utility; his figures for 16,402 person-years exposure show that 35% of the total number of days disability were due to "respiratory disease".

Newman and his colleagues (1938) collected the sickness records for the five years from 1930 to 1934, from 21 boys' schools and 10 girls' schools in Britain; each school had from 200 to 800 pupils, most of whom were boarders. Out of 68,905 pupil-years exposure, there were 50 deaths; 22 of these deaths, that is 44%, were due to meningitis, pneumonia, tonsillitis, influenza or sinusitis with septicaemia, that is to infection with respiratory-tract pathogenic micro-organisms. Of all the time lost due to sickness and trauma, 66% in the case of boy boarders and 73% in the case of girl boarders was due to influenza, infectious fevers and nasopharyngeal infections.

It is concluded that in temperate climates over a fifth of all sickness and death is due to infection with respiratory-tract bacteria and viruses.

(3) Historical Review of Theories and Observations.

Before microorganisms were known to be the cause of infectious diseases, beliefs about the mechanism of spread were based solely upon epidemiological evidence. In the first place, it was realised that certain diseases frequently result from close contact with ill persons. Leprosy was early recognised as a contagious disease, the relationship between contact and infection being readily apparent because prolonged and intimate contact is necessary for transmission of this disease. Sudhoff writes: "Along the Euphrates we come early upon the conception of a chronic, rarely curable disease, characterised by cutaneous changes and capable of transmission to others. Babylonian culture readily drew the proper conclusion and translated knowledge into action. Those affected with the disease must be debarred from intercourse with the healthy. Whoever was defiled by issubbu was banished to the wilderness." (Stubbs and Bligh, 1931).

However, all infectious disease could not be attributed to contact with ill persons. In the case of many epidemic diseases, for instance plague, typhus, small-pox, influenza, cholera, malaria and yellow fever, it could be observed that many persons sickened who had not been in contact with ill persons. In these cases the disease was often attributed to indirect contact through fomites or to air carriage. The causative agent/

agent of pestilential diseases commonly was supposed to be gaseous, being given off in the expired breath or body emanations of the sick person, or in vapours arising from soil, marshes, sewage and other decomposing matter. It seemed probable that such an agent would spread readily through the air. Protection against the disease-causing "miasma" was sought in primitive forms of 'air disinfection', for instance by lighting fires and by fumigation with incense as was practised during the Great Plague of Athens and during the Black Death in the Middle Ages.

In the nineteenth century A.D. the available epidemiological evidence regarding malaria, yellow fever and small-pox, afforded strong support for the view that infection was spread by a 'virus' capable of travelling long distances through out-door air. The epidemiological findings for malaria and yellow fever were explained by the subsequent discovery that infection was insect-borne. However, in the case of small-pox the theory of long-range aerial convection has remained a matter of controversy to the present day (see Chapin, 1912; Millard, 1944 a). Air carriage of the small-pox virus for distances of as much as one mile has been considered the only possible explanation of certain outbreaks which have occurred in the neighbourhood of small-pox hospitals, apparently in the absence of contact with the hospital inmates

(Power/

(Power, 1880-81; Report, 1886-87; Buchanan, 1904; Millard, 1944 a,b). Other authors have attributed the spread of infection in these outbreaks entirely to contact, to contact with incoming patients and to contact with hospital inmates as a result of breaches of hospital discipline (Chapin, 1912; Ware, 1944). This long continued controversy illustrates the inconclusiveness of arguments based on epidemiological evidence and unsupported by direct observation of the natural environmental distribution of the causative microorganism.

Ideas about the nature of infection and contagion, although entertained since biblical times, were vague and indefinite until their clear exposition by Fracastoro (1546). Fracastoro propounded a germ theory of contagious disease; he wrote, "These seeds are the carriers of contagion and that they are the first origin of the disease there can be no doubt. It may be considered that the force of the disease lies in those seeds since they have the power to propagate and reproduce their own kind." He distinguished three mechanisms of spread: infection by contact, infection by fomites and infection at a distance. These doctrines of Fracastoro accord with present-day knowledge, yet because they lacked a basis of experimentally demonstrable facts they did not hasten greatly the progress of sanitary science. Experimental observation became possible after the direct microscopical/

microscopical demonstration of microorganisms by van Leeuwenhoek (1683). The microorganismal causation of many diseases was proved during the later part of the nineteenth century by the work of Pasteur, Lister and Koch; techniques of isolating and identifying the various microorganisms were then developed. These techniques have been used to investigate the mechanisms which are physically capable of transporting micro-organisms from one person to another, the capacity of the causative microorganisms to remain alive and virulent under the conditions of transmission, and the frequency and extent to which, under natural conditions, the different possible vehicles of infection are contaminated with the causative microorganisms.

Most of the early bacteriological investigations related to the spread of pulmonary tuberculosis. Following his discovery of the tubercle bacillus, Koch (1884) demonstrated experimentally that animals readily contracted tuberculosis by inhalation of airborne infected particles; he suggested that under natural conditions such airborne infection might be caused by droplets of sputum discharged in coughing, or by 'dust-particles' derived from pulverisation of sputum dried on floors, clothing and handkerchiefs. Thus arose the theory that respiratory infections are airborne, being spread through the air within dwellings, and thus was initiated the controversy as to whether dust or droplet-spray is the chief agent of airborne infection./

infection. From further experiments, observations and theorisings on the spread of pulmonary tuberculosis, the greater importance has been attributed to "dustborne infection" by Cornet, Chaussé and Lange, and to "droplet-spray infection" by Flügge, Strauss and Wells.

Cornet (1889) demonstrated that living and virulent tubercle bacilli commonly are present in dust on the floor, furniture and beds in hospital wards and private rooms occupied by consumptives; the common occurrence of tubercle bacilli in dust has since been confirmed by many investigators in different countries. Cornet (1899) was able to cause a majority of guinea pigs to contract pulmonary tuberculosis by exposing them in a room to inhale airborne infected dust liberated by the shaking of a sputum-soiled carpet; similar demonstrations of dustborne air infection resulting from the shaking, brushing and beating of sputum-soiled handkerchiefs, cloths and carpets were later reported by Heymann (1901), Chaussé (1913 c,d; 1914 c) and Lange (1926). The likelihood of dustborne infection depends on the capacity of the pathogenic microorganisms to remain alive and virulent under normal room conditions of dryness, light and temperature. Germano (1897 a,b,c) made the first systematic study of the resistance of various pathogenic bacteria to drying; this study and the studies/

studies of many later investigators have shown that several of the respiratory-tract pathogenic microorganisms, including the tubercle bacillus, the diphtheria bacillus, the pneumococcus, the pyogenic staphylococcus, the haemolytic streptococcus, the influenza virus and the small-pox virus, can survive drying and exposure to room-light for a period of some weeks, and so may commonly be spread by dust; other pathogenic microorganisms, particularly those which are Gram-negative such as the cholera vibrio, the plague bacillus, the typhoid bacillus, the influenza bacillus and the gonococcus, were found to be incapable of surviving normal drying for more than a brief period, and so can not commonly be spread by dust.

Flügge (1897) demonstrated that air could be infected by artificial atomisation of a liquid suspension of bacteria; he suggested that air might be infected in natural circumstances by atomisation of respiratory-tract secretions in speaking, coughing and sneezing. Later, Flügge (1899, 1901) suggested that such droplet-spray infection by coughing was the main mechanism of spread of pulmonary tuberculosis; his arguments were based largely on the experimental work of his pupils Laschtschenko, Heymann, Sticher, Beninde and Möller. Laschtschenko (1899) proved that many small saliva droplets are expelled from the mouth in speaking, coughing and sneezing; he inoculated the mouth/

mouth secretions with B.prodigiosus as a "marker", and exposed nutrient agar plates in various parts of the room to reveal the fall of droplets containing this "marker" bacillus. Some droplets were found to be distributed to all parts of the room, a few even to a distance of 6 meters. Laschtschenko (1899) and Heymann (1899, 1901) showed that tubercle bacilli were present in a small number of the droplets expelled by the coughing of consumptives; the great majority of these infected droplets fell out of the air rapidly and within a short distance, but occasionally a few of the infected droplets were found to remain suspended in the air for periods of up to 1 hour. Flügge (1899) concluded from these experiments that tuberculosis would rarely be contracted except by persons who expose themselves within arm's length in front of a patient during a paroxysm of coughing; he wrote, "When we try to draw practical conclusions concerning droplet infection from the results of the experiments, then one has to admit above all, that a person, by the fact that he is in the neighbourhood of a coughing consumptive, can inhale tubercle-bacillus-containing droplets which have been distributed into the air by the consumptive during the coughing. But the experiments teach us at the same time under which conditions and with what limits this kind of infection can occur. In the first place, by no means all consumptives spray droplets. Individual variations, the/

the different numbers of bacilli in the sputum, the time of day, etc., play a part in this respect. Many consumptives do not seem to spray anything at all; others only during a certain disease period; many only at a certain time of day. In the second place, the distance of the inhaling person from the coughing patient is important. Up to 50 cm. quite heavy spraying occurs; further than this the number of floating droplets decreases enormously in accordance with the distribution of the discharged breath in all directions of the air space. At 1.5 meters the microscope slides usually remain sterile. However, one may not conclude from this that no bacteria occur in the more distant air-zones, since the aspirator experiments show that eventually some may be demonstrated, but their dilution is so great that the chances of infection are negligible."

Flügge and his followers based their view that droplet-spray is a more important vehicle of infection than dust, partly on arguments that the tubercle bacillus can not survive long enough the conditions of drying which are necessary before sputum can be reduced to dust, and partly on comparison of the amount of airborne infection produced by coughing in the experiments of Laschtschenko (1899) and Heymann (1901), with the amount produced by dust-raising from sputum-soiled handkerchiefs and carpets in the experiments of Heymann/

Heymann (1901), Sticher (1899) and Beninde (1899). It may be remarked that the results of these experiments were by no means clearly indicative of the greater importance of droplet-spray; the present writer agrees with Chaussé (1916) who, when reviewing the work of Flügge's pupils, wrote: "These young experimenters, being charged with confirming the published views of their master, obviously forced their conclusions to the sense that was favorable to him". However, in spite of the work of Chaussé (1912, 1913 a, b, c, d; 1914 a, b, c; 1916) and Lange (1926), which affords strong reasons for believing that droplet-spray is a less important agent of infection than sputum-dust, for some reason, possible because it is impressive and dramatic, the theory of droplet-spray infection has continued to command an undue respect from the medical profession, sometimes even to the extent that the more likely means of infection are forgotten.

Following the early preoccupation with pulmonary tuberculosis, greater attention was given to the other respiratory infections. Up to the present time, observations have been recorded of the expulsion in droplet-spray by infected persons of Strept.pyogenes, Cor.diphtheriae, N.meningitidis, H.pertussis, Monilia albicans, Past.pestis, Myco.leprae and the spirochaetes and fusiform bacilli of Vincent's angina, each usually in very small numbers. Correspondingly, many/

many observations have been recorded of the presence in dust on the floors, bedding and clothing of infected persons, of Strept.pyogenes, Cor.diphtheriae, Dip.pneumoniae and Staph.aureus, often in very large numbers.

At the beginning of the twentieth century it was discovered that in the case of many respiratory infections, not only sick persons could act as a source of infection, but also certain healthy persons, "carriers" of the pathogenic microorganism. Thus it became possible to attribute to contact with an unknown "carrier" all those frequently observed cases of infection which have no history of contact with a known sick person. Previously, such cases had been thought explicable only as the result of infection by fomites or by air carriage. Now, it was no longer necessary to suppose that infection could be spread by any means other than by immediate contact. Many medical authorities came to hold the view that contact was the chief mode of infection. Chapin (1912) wrote: "Contact infection is the most obvious mode of transmission of the infectious diseases. For the sick to touch the well, and thus to infect them, seems to be the most natural way of accounting for the spread of these diseases. If contact infection can explain the epidemiological phenomena, there is no occasion for assuming the growth of pathogenic germs outside the body/

body, or infection by fomites, or infection by air, or any other similar theory, and no such theory should be adopted as a working hypothesis unless pretty strong evidence can be brought to its support." Chapin allowed that infection might occasionally be spread by fomites, by droplet-spray or by dust-particles, but he regarded transmission by such vehicles as infrequent because of the tendency of pathogenic microorganisms to die or lose their virulence when cast off from the body. Chapin supported his views by reasoning from epidemiological observations. If infection can be spread through the air, one may suppose that all persons must become infected who breathe the air of rooms occupied by patients. But this does not happen. In illustration, Chapin quoted the reported success of certain hospitals in preventing cross-infection by the taking of precautions against contact infection, and observations that scarlet fever and diphtheria do not spread, in the absence of close contact, from one family to another living in different apartments of the same house, or from patients to visitors in fever hospitals. Thus, "of three hundred to four hundred non-immune students who visited the scarlet fever wards of the Philadelphia hospital, remaining in the ward for twenty minutes to an hour, not one contracted the disease" (Welch and Schamberg, 1905).

A new appreciation of the epidemiology of the respiratory infections was brought about by the knowledge/

knowledge gained in the succeeding two decades about the high frequency of subclinical and "carrier" infection, and of the immunity resulting from such infection. It is realised now that most adults possess considerable immunity to such infections as scarlet fever, diphtheria, pneumococcal pneumonia and meningococcal meningitis, although not previously having suffered the clinically-manifest infection; if they acquire the pathogenic microorganism, these persons tend to become healthy carriers rather than diseased patients. The epidemic of clinically-manifest disease is known to be only a small reflection of the much greater underlying "carrier epidemic". Thus, when low attack rates are recorded, these must not be taken as a certain indication of low infectivity. If the frequency of subclinical, as well as clinical infection, is taken into account, it is found that the respiratory infections are characterised by a high incidence, approaching universality, and a very great facility of spread. Chapin (1925), modifying his previous views, wrote: "Scarlet fever, consequently, is in reality much more contagious than the diagram would indicate, for the number of non-immunes is less than formerly assumed. It therefore seems probable that the greater contagiousness of measles --- is to a considerable extent apparent only. --- Indeed, it is possible that diphtheria might be proved to be not so very much less contagious than measles in this respect."

The/

The past decade has brought the concept of "the dynamic spread" of respiratory infections, with indoor air postulated as a common source medium of infection (Wells and Wells, 1936; Wells, Wells and Mudd, 1939; Wells, 1944). These authors argue that it is necessary to assume that the respiratory infections are airborne in order to explain their high incidence and great facility of spread, their seasonal periodicity which apparently is due to the variation of domestic ventilation with outdoor temperature (Wells, 1944), the success of air disinfection in reducing their incidence in schools and convalescent homes (Wells, Wells and Wilder, 1942; Harris and Stokes, 1945), and the success of isolation in single-bedded wards, as contrasted with the failure of aseptic bed-isolation nursing in multi-bed wards, in preventing the occurrence of hospital cross-infections (Allison and Brown, 1937; Wright, Shone and Tucker, 1941; Stalker, Whatley and Wright, 1942).

Strong support has been afforded to the belief that indoor air is an important vehicle of infection, by numerous observations of the presence of living pathogenic respiratory-tract bacteria in the air of private rooms and hospital wards occupied by infected persons: of Myco.tuberculosis, Strept.pyogenes, Cor.diphtheriae, N.meningitidis, Staph.aureus, H.influenzae, Dip.pneumoniae and Monilia albicans. Generally/

Generally, the numbers of pathogenic bacteria found in the air have been small, both absolutely and relatively in comparison with the numbers of saprophytic bacteria. The first common method of examining the bacterial content of air was collection of the sedimenting particles on uncovered culture plates. This method is very simple and easy to carry out, and accordingly has been much employed. However, it is not a satisfactory method of air sampling for the reasons that it does not give a definite quantitative measurement of the amount of air infection, and that it reveals only the large infected particles which settle rapidly out of the air. Many instruments have been developed for accurate quantitative examination of the bacterial content of air, the most widely used being the "air-centrifuge" of Wells (1933) and the "slit sampler" of Bourdillon, Lidwell and Thomas (1941). Dr.Lidwell's modification of the "slit sampler", which is capable of making a continuous 24-hour record of the course of the air infection, undoubtedly is the finest air sampler available.

At the present time it is widely accepted that <u>airborne infection</u> is an important mode of spread, if not the most important mode of spread, of the respiratory infections. There is less agreement about the manner in which the air becomes infected. Two ways are suggested whereby pathogenic microorganisms may/

may enter the air, in droplet-spray which is produced by speaking, coughing and sneezing, and in dust which is liberated as a result of friction and movement from the skin and clothing of infected persons, from their bedding, or from the floor and furniture of their rooms.

The pioneer work of Flügge and his pupils on droplet-spray had suggested that the air infection which is produced by speaking, coughing and sneezing, is of only brief duration and localised within a few feet of its source. The experimental observations of Chaussé (1913 a,b) and Lange and Keschischian (1925), and the calculations of Wells (1934) have shown that this view is not necessarily correct. Chaussé (1913 a) measured the colored particles falling at different times out of air in which an aqueous dye-solution had been atomised; he found that droplets larger than 200 microns in diameter had all fallen out of the air within a few seconds after atomisation, but that the smaller droplets, evaporating almost at once to form dry residues of much smaller size, could remain suspended in the air for up to 7 hours. Lange and Keschischian (1925) confirmed Chaussé's observations. Wells (1934), apparently without knowledge of this earlier work, reached the same conclusions on theoretical grounds. He showed that droplets smaller than 100 microns, instead of falling to the ground within a few seconds after expulsion from the mouth, will/

will at once evaporate to form minute solid residues which will be small enough to be capable of remaining airborne for several minutes to several hours, and thus of being carried by air currents far throughout a room or building; Wells named these solid residues "droplet nuclei". As any pathogenic microorganisms present in the parent droplets will remain in the droplet-nuclei, the droplet-nuclei may constitute an agent of widespread aerial infection. That such droplet-nucleus infection plays a major part in the spread of respiratory infections, has been argued by Wells and his colleagues in a very extensive series of articles published during the last fifteen years. It has been demonstrated that large numbers of droplet-nuclei are produced by speaking, coughing and sneezing (Wells and Wells, 1936; Jennison, 1942; Bourdillon, Lidwell and Lovelock, 1942). However, the production by infected persons of droplet-nuclei which contain pathogenic microorganisms, has not been demonstrated except rarely and doubtfully; airborne "droplets" (droplet-nuclei) containing tubercle bacilli were found to be produced by the coughing of consumptives on a few occasions and in small numbers (Laschtschenko, 1899; Heymann, 1899, 1901; Chaussé, 1914 a,b, 1916; and Hippke, 1921); an attempt to demonstrate the production by coughing of droplet-nuclei containing haemolytic streptococci, was unsuccessful (Hare, 1940). Hare/

Hare has suggested that pathogenic bacteria such as the haemolytic streptococcus, when present in droplet-spray, are contained exclusively in the large droplets which fall at once to the ground, failing to become droplet-nuclei.

Intensive studies have been made in recent years of the occurrence of dustborne infection of air, and much convincing evidence has been gathered to show that air is readily infected with dustborne pathogenic microorganisms. The observation by Chaussé (1914 c), in an unoccupied tuberculosis ward, of the natural occurrence of air infection with dustborne tubercle bacilli, has now been followed by observations that, under natural conditions in hospital wards, air infection with Strept.pyogenes and Cor.diphtheriae varies in definite relation to the concurrent dust-raising activities such as sweeping and bed-making (White, 1936; Cruickshank, 1941; van den Ende and Spooner, 1941; Crosbie and Wright, 1941). Oiling of floors and bedding has been practised as a means of preventing the liberation of dust into the air; the reported success of oiling in reducing the frequency of respiratory infections in hospitals and barracks, affords strong evidence that airborne dust plays an important part in spreading the infections (Wright, Cruickshank and Gunn, 1944; Dingle et al., 1946).

At the present time there is not any generally accepted/

accepted view as to the relative importance of dust and droplet-nuclei in causing air infection. Wells, Winslow and Robertson (1946) draw a sharp distinction between dustborne air infection and droplet-nucleus air infection according to particle size and duration of air carriage: droplet nuclei are small, remain airborne for long periods, are dispersed widely indoors, can penetrate to the lung alveoli, and cause epidemic infections; infected dust-particles are large, settle rapidly giving only brief and localised air infection, and cause endemic infections of the nose and throat. The validity of this distinction is not generally accepted. Available data on the size of infected dust-particles and droplet-nuclei is not sufficient for a definite conclusion to be drawn.

(4) Sources of Respiratory-Tract Pathogenic Bacteria.

A "source" of pathogenic microorganisms is a situation in which the microorganisms are multiplying, a focus in the body of their host. This is to be distinguished from a "reservoir" of pathogenic microorganisms, which is an external object or surface, such as the hand, handkerchief, clothing or floor, where many of the microorganisms are collected, and survive for some time, but where they do not undergo multiplication.

In the majority of respiratory infections the main sources of the causal microorganisms are the throat, the nose and the mouth of patients and "healthy carriers". The case of lung and bronchial infections will be considered separately, in relation to pulmonary tuberculosis. Pathogenic respiratory-tract bacteria may originate from colonised regions of the body outside the respiratory tract, including the healthy skin, skin sores, infected burns and infected wounds. Certain pathogenic respiratory-tract bacteria may originate from domestic animals, particularly in cow's milk.

Both in patients and in "carriers", the throat, including the tonsils, oropharynx and nasopharynx, is much the commonest site harbouring pathogenic bacteria. However, microorganisms are disseminated into the environment much less abundantly from the throat than from the front of the mouth or from the nose. Great interest thus attaches to the frequency of/

of occurrence of pathogenic microorganisms in these latter situations.

The special importance of the anterior mouth as a source of infection, as compared with the throat, is apparent from the finding that the secretion-spray expelled in speaking, coughing and sneezing is derived mainly from the front parts of the mouth and only to a small extent directly from the throat (Bloomfield and Felty, 1924; Jennison, 1942).

The special importance of the nose as a source of infection has emerged from the work of Hamburger and his colleagues, who have shown that haemolytic streptococci are dispersed into the environment much more readily and abundantly from the nose than from the throat. The existence of specially dangerous carriers having an exceptional streptococcal output, was suggested by the finding of Hamburger (1944 a) that, in multi-bed hospital wards for infectious fevers and "common respiratory disease", cross-infections by haemolytic streptococci sometimes spread rapidly when as few as 1 or 2 carriers (up to 12%) were originally present, and sometimes were absent when over 50% of the patients harboured group-A streptococci; that the cross-infection rate varied with the numbers of streptococci distributed into the environment rather than with variations in strain virulence or host resistance, was suggested by the finding of Hamburger, Puck, Hamburger and Johnson (1944) that when there were cross-infections with /

with more than one serological type of streptococcus, the number of cross-infections due to each type bore a rough but definite relationship to the number of streptococci of that type found to be present in the ward air during the outbreak. Hamburger, Green and Hamburger (1945 a) found that carriers with strongly positive nose cultures were especially "dangerous"; in an examination of 400 hospitalised patients with haemolytic streptococcal infection of the upper respiratory tract, it was found that on average about 80 times as many haemolytic streptococci were expelled per day on to their bed-linen (i.e. on to a 25-sq.in. bottom-sheet "patch") by patients with strongly positive nasal cultures (241) as by patients with positive throat cultures but negative or weakly positive nasal cultures (163); the haemolytic-streptococcal content of the air of a ward containing patients with positive nasal cultures was found to be about 35 times higher than that of the air of a ward containing patients with only the throat cultures positive. Hamburger, Green and Hamburger (1945 b) used the serological typing method to trace cross-infections by haemolytic streptococci and found that nasal carriers were much the commonest source of such infections. Of 12 hospital cross-infections, 11 originated from patients with strongly positive nasal cultures and only 1 from a patient with a negative nasal culture; the streptococcal output of 7 of the infecting nasal carriers was observed and in all cases /

cases found to be very great (e.g. 40,000 to 400,000 per 25-sq.in. sheet "patch" per day); the sole infecting carrier with a negative nasal culture had an unusually large number of haemolytic streptococci, 1,000,000 per ml., in his saliva, but was not found to distribute any streptococci on to his bedding. Of 16 soldiers from 7 barracks who were admitted to hospital with streptococcal infections, 15 came from barracks in which there was a nasal carrier of the infecting type, in all but one case giving a strongly positive nasal culture, and only 1 soldier-case came from a barrack where the sole possible source of the infecting type was a throat carrier with a negative nasal culture; among the 347 men resident in the 7 barracks, group-A streptococci were carried in the nose as well as in the throat by 20 men, of whom 12 harboured a cross-infecting type, and by a further 47 men in the throat only, of whom 12 again harboured a cross-infecting type. A hospital-wide foodborne epidemic of 106 cases of pharyngitis-tonsillitis due to a type-1 streptococcus was traced to a "cold-food handler" having a profuse nasal discharge, strongly positive nose and throat cultures of the type-1 streptococcus, a high output of streptococci into his environment (sheet "patch" test) and contamination of his hands to the extent of over 10,000,000 streptococci. Hamburger and Green (1946) found that on average the hands of a nasal carrier, of whom 106 were examined, bore about 170 times as many haemolytic streptococci /

streptococci as the hands of a throat carrier having a negative nasal culture, of whom 74 were examined. Confirmation of Hamburger's theory about "dangerous nasal carriers" is to be found in the earlier work of Hare (1941) who swabbed various parts of the body and clothing of streptococcal carriers and found that in the 3 nasal carriers studied "the contamination of the person was more widespread and persisted longer than in the remainder who had the organisms in the throat only" (i.e. 4 throat carriers).

It is apparent that the frequency with which pathogenic microorganisms are present in the nose and anterior mouth of infected persons is of much greater significance than the frequency of "throat carriage". The throat, especially the tonsils and nasopharyngeal lymphoid tissue, undoubtedly is the optimal habitat of pathogenic bacteria such as the haemolytic streptococcus, the pneumococcus and the meningococcus, allowing free multiplication; the frequency of occurrence of these pathogenic bacteria in the throat is much greater than the frequency of their occurrence in the nose or in the anterior mouth. Bloomfield (1921 a,c; 1922 b) showed that, in carriers, colonisation by the pathogenic bacteria usually is strictly confined to one particular locality within the respiratory tract, probably a focus of diseased tissue. Bloomfield (1921 a) studied two "persistent carriers" of Friedlander's bacillus and found that while the bacilli always were present in large

numbers /

numbers on swabs from one tonsil, they were present only occasionally and only in small numbers on swabs from the pharynx and the nose (e.g. in the right and left nares on 1 of 6 occasions). Bloomfield (1921 c) observed other "persistent carriers": a carrier always bearing many Staph.aureus in the nose (anterior nasal mucosa) who did not on any occasion show this staphylococcus in other localities; a carrier always bearing many Staph.aureus on the tonsils who only occasionally showed a few of the staphylococci in the pharynx and never any on the tongue or in the nose; and two carriers always bearing many beta-haemolytic streptococci on both tonsils and in the pharynx, who did not on any occasion show the streptococci on the tongue or in the nose (except that one carrier showed a few in the nose on 2 out of 8 occasions). Bloomfield (1922 b) observed three persistent carriers of H.influenzae: two pharyngeal carriers who on each two occasions showed many influenza bacilli in the pharynx, but none on the tonsils, soft palate or tongue; and a tonsillar carrier who on each of three occasions showed many influenza bacilli on both tonsils, but none in the pharynx or on the tongue. Other similar observations on the carriage of the influenza bacillus were recorded by Bloomfield (1921 d; 1922 c).

This strict localisation of pathogenic bacteria to certain regions within the respiratory tract was found /

found to apply in the case of patients with acute infection as well as in the case of healthy carriers; Bloomfield and Felty (1924) studied on one occasion a patient with acute tonsillitis who showed very many haemolytic streptococci on his tonsils and pharynx, but none on his lips, tongue, and right and left buccal mucosae.

The anterior mouth, including the tip of tongue, sublingual space, front gums and inner surfaces of the lips, is not a favourable region for colonisation by the pathogenic bacteria. It is unlikely that any multiplication takes place in the anterior mouth. The front parts of the mouth may, of course, become contaminated with bacteria from the throat by speaking, coughing, sneezing, hawking, spitting and chewing, and perhaps also with bacteria from the nose by licking the upper lip after its soiling with nasal secretion; pathogenic bacteria found in the anterior mouth almost certainly will have originated in this way from the nose or throat. Bloomfield and Felty (1924) examined the anterior mouth secretions of acute tonsillitis patients by making them kiss a culture plate and also by culturing from a spatula which had been placed for several minutes in the front of the mouth; each of two patients who yielded many haemolytic streptococci on throat swabs, initially showed complete absence of haemolytic streptococci from the anterior mouth, but, after they had hawked and spat, one of the two patients showed haemolytic /

haemolytic streptococci in the anterior mouth secretions, one colony on the kissed plate and many from the spatula. Similarly, one out of three normal persons whose throats had been inoculated with E.coli, contaminated his anterior mouth with this bacillus as a result of hawking and spitting. Such contamination of the anterior mouth is only temporary and must disappear unless repeatedly renewed. The eliminative mechanisms of the mouth include the backwards flushing by saliva flow and by eating, and the antibacterial action of the saliva towards certain pathogenic bacteria such as the meningococcus, the diphtheria bacillus and the tubercle bacillus; these mechanisms remove bacteria from the mouth within ¼ to 24 hours; (Sanarelli, 1891; Miller, 1903; Barnes, 1907-09; Gordon, 1916; Bloomfield, 1919, 1920, a,b,c; Bloomfield and Huck, 1920; van der Reis, 1921; Bézi, 1932; Dold and Weigmann, 1934; Dold, 1935; Arnold and Stuart, 1935; Hood and Arnold, 1937; Weigmann and Noeske, 1937; Casassa, 1937; Appleton, Klein and Palmer, 1938; Kanter and Appleton, 1940; Weigmann and Holzl, 1940; Holzl, 1941; Appleton, 1944; Thompson and Shibuya, 1946).

The nasal cavity, when its lining mucosa is in a healthy state, is not a favourable site for colonisation by bacteria. However, pathogenic bacteria may enter it from the nasopharynx during sneezing and other expiratory activities; for instance, /

instance, Bloomfield (1921 c) found beta-haemolytic streptococci to be present at 2 out of 7 occasions on the anterior nasal mucosa of a persistent throat-carrier of this bacterium. The bacterial intruders from the nasopharynx are rapidly removed or destroyed; bacteria are removed from the healthy nasal cavity within ¼ to 24 hours by eliminative mechanisms which include the backwards flushing of the mucus film on the ciliated epithelium, phagocytosis by emigrant leucocytes, and a bactericidal action due to lysozyme in part (Thomson and Hewlett, 1896; Calvino, 1899; Bloomfield, 1919, 1920 a, b, c; Arnold, Ostrom and Singer, 1928; Hilding, 1932 a,b; Linton, 1932; Daly, 1938). In any case, not much nasal secretion is discharged from the healthy nose, so that a profuse distribution of infection from the nose is not likely to result from such temporary contamination of it; indeed, Hamburger, Green and Hamburger (1945 a) found that haemolytic streptococci were not distributed in particularly large numbers by carriers with weakly positive nasal cultures. Active colonisation of the nasal mucous membrane by pathogenic bacteria such as the haemolytic streptococcus and the diphtheria bacillus may occur under certain circumstances, probably when the eliminative mechanisms are impaired due to an unhealthy state of the mucous membrane; in such cases, with the pathogenic bacteria multiplying freely in the nasal cavity and with an excess of nasal /

nasal secretion being discharged, the nose is very likely to be an important source of infection and to distribute very large numbers of pathogenic bacteria into the environment. That colonisation of the nasal mucous membrane commonly causes local inflammation and nasal discharge, has been shown by Lemon and Hamburger (1946) in the case of apparently healthy "nasal carriers" of group-A haemolytic streptococci; 109 ambulatory nasal carriers of this streptococcus were discovered in nose-culture surveys among soldiers not reporting sick, or, in a few cases, reporting respiratory symptoms at sick parade but not absenting from duty; 84 (i.e. 77%) exhibited an unusually high serum anti-streptolysin response together with manifest inflammation of the pharynx, or nasal discharge, or a history of sore throat, and thereby were classified as "missed cases"; 18 (i.e. 17%) were unclassifiable, and only 7 (i.e. 6%) could be classed as true "contact carriers" in which colonisation seemed harmless and the streptococcus to act like a commensal parasite.

Bloomfield (1921 b,c) in studies of the bacteria occurring in the upper respiratory tract of healthy persons, differentiated a constant normal flora of commensal bacteria always present in large numbers, a persistently-carried occasional flora of certain bacteria (including the haemolytic streptococcus, the pneumococcus, the influenza bacillus, Friedlander's bacillus and Staph.aureus), and a transient /

transient flora of bacteria introduced by chance from the air, nostrils, skin and other external sources, and surviving for a short time before being removed or destroyed by the eliminative mechanisms; as transient intruders of this latter kind, saprophytes such as Staph.albus, Micrococci and A.aerogenes were found commonly, but pathogens such as the haemolytic streptococcus and Staph.aureus were also found quite frequently. De Waal (1941) showed that the nose and throat of a non-infected person who enters the room of an infected person, very readily and frequently become contaminated temporarily with haemolytic streptococci inhaled from the room air, and rid themselves of these streptococci within a day of acquisition. These slight and brief contaminations with pathogenic bacteria, the "transient flora", can not represent an important source of infection for other persons; the "temporary carriers" can not be very dangerous. Yet, carrier-rate statistics obtained by mass throat- and nose-swabbing, must include these "temporary carriers" along with the true "persistent carriers", which latter, breeding the pathogenic bacteria in their respiratory tract, alone represent important sources of infection; this shortcoming must be taken into account when assessing the significance of reported "carrier rates". The natural duration of infection in "persistent carriers" varies from a few weeks to a few years, commonly being a few months. Bloomfield (1921 a) examining four /

four throat-carriers of Friedlander's bacillus, found that they remained infected for over 36 days, 42 days, 3 months and 4 months respectively. In an extensive study of the nose and throat floras of healthy persons in South-East England, it was found that carriers of the pneumococcus remained infected with a particular type for a few months in many cases and for a few years in some, and that carriers of the meningococcus remained infected for a short period or, occasionally, for up to 6 years (Report, 1939).

Carriage of Strept.pyogenes.

The great majority of patients in the acute stages of scarlet fever or tonsillitis yield haemolytic streptococci from the throat: Green (1937), taking single throat-swabs, obtained positive cultures from 1581 (i.e. 84.3%) of 1875 patients with acute scarlet fever; de Waal (1940) obtained positive cultures from 1664 (i.e. 96.6%) of 1831 scarlet fever patients.

After clinical recovery, a proportion of these patients continue, as "convalescent carriers", to harbour the haemolytic streptococcus for some weeks or months. Williams, Hussey and Banzhaf (1924) found that after 30 days in hospital, 20% of convalescents from scarlet fever still were carriers. Gunn and Griffith (1928) found that haemolytic streptococci of the original infecting type still were present in the throat or nose at the time of discharge /

discharge from hospital (on average after 7 weeks) in the case of 20 out of 50 scarlet fever patients; the average time to clearing of the throat was 3¼ weeks in the case of the other 30 patients. Kirkbride and Wheeler (1930) found that 50% to 60% of convalescents were carriers after 30 days. Brown and Allison (1935) found that 83% of all patients still carried the streptococcus in throat (76%) or nose (33%) at the end of hospitalisation. Green (1937) found that the original infecting type of haemolytic streptococcus was still carried at the time of discharge from hospital, by 34.9% of 1062 convalescents from scarlet fever. De Waal (1940) found that the original infecting type of streptococcus persisted throughout the whole period of residence in hospital in the case of 6 out of 25 scarlet fever patients who were swabbed at weekly intervals.

Much more numerous and more important in the spread of infection than patients and convalescent carriers, are the "healthy carriers", including "contact carriers" who have had known contact with a patient, and "non-contact carriers" who have not had known contact with a patient but have acquired the haemolytic streptococcus from another carrier. The proportion of a population comprising healthy throat-carriers of Strept.pyogenes usually is between 1% and 10%. The carrier rate may, however, range widely beyond these limits in the case of particular small population /

population groups. The carrier rate tends to be high in temperate climes, in the winter and spring, in the poor, ill-nourished and overcrowded sections of the community, among children and adolescents, and, above all, in residential institutions such as training camps and boarding schools with multi-bed dormitories, where "carrier epidemics" may involve over 50% of the inmates. The carrier state appears to be temporary and universal, in that probably every person becomes a carrier of the haemolytic streptococcus for a few weeks or a few months, at least once every few years (Report, 1939). Many carrier surveys have been made for infection by haemolytic streptococci, regardless of whether these belonged to group A, that is, were Strept.pyogenes, the species which is pathogenic to man: the haemolytic-streptococcus throat-carrier rates reported, include 26% in Puerto Rico (Pomales, 1929), very low in Alabama and Labrador (Burky and Smillie, 1929), 17% in New York (Dochez, Shibley and Mills, 1930), 0% to 36% in Manchester (Report, 1930), 1.7% to 4% in St. John, U.S. Virgin Isles (Milam and Smillie, 1931), 5% among Eskimoes (Wells and Heinbecker, 1932), 6% to 13% in New York (Bourn, Carpenter and McComb, 1933), 0.3% in Spitzbergen (Paul and Freeze, 1933), 17.5% among children admitted to a children's hospital (Peacock, Bigler and Werner, 1939), and 0% to 54%, mainly 5% to 20%, in London and South-East England (Report, 1939).

Hare /

Hare (1935) found that only about a third of such throat-carrier strains of beta-haemolytic streptococci belonged to group A (63 of 100 strains producing soluble haemolysin belonged to group A). Hare (1940 b) reviewed "grouping" observations made by various investigators on haemolytic-streptococcus strains found in the throats of healthy persons; a total of 574 haemolytic-streptococcus strains recovered among 3102 persons, included 216 group-A strains, 15 group-B strains, 84 group-C strains, 1 group-D strain, 19 group-F strains, 74 group-G strains, 27 group-H strains, 8 group-K strains and 11 other strains; that is, 18.5% of the persons carried beta-haemolytic streptococci, but only 7% carried beta-haemolytic streptococci belonging to group A (i.e. Strept. pyogenes). Williams and Harper (1944), in routine swabbing of normal throats of patients and nursing staff in a surgical ward of the Birmingham Accident Hospital, found that among 1553 cultures from 532 persons, 43.5% contained haemolytic streptococci and 19.2% haemolytic streptococci of group A; of the haemolytic streptococci, 44.2% belonged to group A, 0% to group B, 1.6% to group C, 0.5% to group G and 53.7% to other groups. Hodes, Schwentker, Chenoweth and Peck (1945) reported group-A streptococcus throat-carrier rates varying from 4% to over 60% among 400 recruits in an American naval training centre.

Little information is available about the frequency /

frequency with which haemolytic streptococci are are present in the anterior mouth of patients and carriers. Presence of haemolytic streptococci in saliva from the mouth as a whole was observed in 12% of healthy persons in Manchester (Report, 1930). Hare (1940 a) found that haemolytic streptococci were present on some occasions in the saliva of 7 out of 9 convalescents and throat-carriers. Hamburger (1944 b) found that haemolytic streptococci were present in 341 (i.e. 64.6%) of 527 saliva samples from 156 patients with scarlet fever, tonsillitis or pharyngitis, who showed haemolytic streptococci in throat cultures; in the various positive saliva samples the number of haemolytic streptococci varied from 100 to 5,000,000 per ml., being in most cases between 1000 and 1,000,000 per ml.

Rather more information is available about the frequency with which haemolytic streptococci are present in the nose of patients and carriers. Among patients, nasal infection with haemolytic streptococci was found in 124 (i.e. 30%) out of 415 persons with scarlet fever (de Waal, 1940), and in about two-thirds of persons with acute streptococcal tonsillitis or pharyngitis, or with scarlet fever (Hamburger, Green and Hamburger, 1945 a). Among healthy persons, "nasal carriage" of haemolytic streptococci has been observed in 0.05% of examinations of persons in Manchester whose throat-cultures included 8% which were positive (Report, 1930), in 0.4% of persons in New /

New York of whom 17% were throat-carriers (Dochez, Shibley and Mills, 1930), in 5.4% of doctors and nurses of whom 20 to 30% were throat-carriers (White, quoted by Hare, 1935), in only a very small proportion of persons in London and South-East England (Report, 1939), and in 4.5% of children entering hospital of whom 17.5% were throat-carriers (Peacock, Bigler and Werner, 1939); "nasal carriage" of haemolytic streptococci belonging to group A has been observed in 2.1% of examinations of persons in Birmingham whose throat-cultures included 19.2% containing group-A streptococci (Williams and Harper, 1944), and in 4.3% of healthy soldiers of whom 19.1% were throat-carriers of the group-A streptococcus (Hamburger, Green and Hamburger, 1945 a).

Carriage of Cor.diphtheriae.

The diphtheria bacillus is present abundantly in the throat secretions of most patients in the acute stage of faucial diphtheria; Graham-Smith (1903), reviewing previous observations in Europe and America, reported that diphtheria bacilli had been found in 72% of 27,000 certified cases.

After clinical recovery a proportion of the patients continue, as "convalescent carriers", to harbour the diphtheria bacillus in the throat. Graham-Smith (1902) found that the mean duration of "convalescent carriage" of the bacillus was 28 days from the date of notification; exceptionally, carriage continued up to 87 days. Wright (1941), taking /

taking weekly swabs from the nose and throat of 311 diphtheria patients originally yielding positive cultures, found that the diphtheria bacillus was still present after 1 week in 68% of the patients, after 2 weeks in 55%, after 3 weeks in 44%, after 4 weeks in 32%, after 5 weeks in 23%, after 6 weeks in 18%, after 7 weeks in 13%, after 8 weeks in 9%, after 9 weeks in 7%, and after 10 weeks in 5%.

A big proportion of the "healthy contacts" of patients have been found to harbour the diphtheria bacillus in the throat. Park and Beebe (1894) found that virulent diphtheria bacilli were carried by 50% of 48 children from 14 infected families, and by 9% of 55 children from an institution where there were occasional cases. Graham-Smith (1903) reviewed reports of "contact-carrier rates" which ranged from 3% to 100%. Chapin (1912) reviewed numerous observations made in Britain and America, of the diphtheria-bacillus carrier rate among well persons having contact with patients in schools or families; these carrier rates ranged from 10% to 50%, being mostly between 10% and 20%.

The diphtheria bacillus is carried in the throat by a much smaller proportion of "healthy non-contacts", persons not known recently to have been in contact with the disease. Graham-Smith (1903) cites four previous investigations by other authors; out of a total of 3374 healthy non-contacts examined, 0.2% carried virulent diphtheria bacilli. In his own

investigation/

investigation of 362 persons, Graham-Smith (1903) did not find any person carrying virulent bacilli. Guthrie, Gelien and Moss (1920) in two throat examinations of 800 school children found virulent diphtheria bacilli in about 1% (i.e. diphtheria bacilli in 10.6% and 8.6%, of which strains only 12% were virulent). Dudley (1931), examining apparently-healthy boys of $11\frac{1}{2}$ to $15\frac{1}{2}$ years who were resident in Greenwich Hospital School between 1922 and 1930, found that the mean throat-carrier rate for diphtheria bacilli was 6.9%, and for toxigenic diphtheria bacilli was 2.9%; the duration of infection of these "healthy carriers" rarely exceeded 2 weeks. Christison, Wright and Shearer (1936), examining 3429 swabs from normal adults and school children in Edinburgh, found a throat-carrier rate of 1.3% for diphtheria bacilli, and of 0.5% for virulent diphtheria bacilli. Murray (1943), examining Bantu children in Bechuanaland, observed the carrier rate for virulent diphtheria bacilli to be 3.2% among 499 rural children and 1.8% among 437 urban children. A review has been given (Report, 1945) of various recent observations of the carrier rate for virulent diphtheria bacilli among immunised children; the rates given were 0.38% and 0.19% in London, 0.03% in Kingston, N.Y., 0.04% in Toronto, 0.5% in Virginia, 0.56% in Cleveland, Ohio, and 0.87% in Alabama, Cooper (1945), investigating a closed and crowded community which had been fully immunised, found that virulent /

virulent diphtheria bacilli were carried in the throat by 42% of 94 boys, 11% of 64 girls and 0% of 11 adults.

Little is known about the frequency of occurrence of diphtheria bacilli in the anterior mouth. Teague (1913) found the bacilli in expectorated saliva from 18 of 27 patients. Strauss (1922) found the bacilli in mouth-swabs (of tongue, front teeth and gums) from 2 of 5 patients and 0 of 3 carriers with infected tonsils.

Virulent diphtheria bacilli frequently are present in the nose, in a proportion of patients with faucial diphtheria, in patients with nasal diphtheria, and in healthy carriers. Ravenel (1895) found diphtheria bacilli in the nose of 33 out of 41 patients with membranous rhinitis. O'Meara (1931) found the diphtheria-bacillus nasal-carrier rate to be 3.2% among 1000 healthy Dublin school-children who showed a throat-carrier rate of 2.5%. Boissard and Fry (1941, 1942) found diphtheria bacilli in nasal swabs from more than a third of 388 patients with faucial diphtheria, and in nasal swabs from 19 (i.e. 8.2%) of 231 normal children examined at the time of an epidemic.

Discharge from the ear of persons suffering from diphtheritic otitis has been emphasised as possibly being an important source of infection, by Chapin (1912) and by O'Meara (1931).

Carriage of Dip. pneumoniae.

Pneumococci /

Pneumococci of the infecting type are present very commonly in the throat of patients with pneumococcal pneumonia. They remain usually for only a few days, but they may persist for several months or even for over 2 years in the "convalescent carrier" (Cruickshank, 1933).

A large proportion of healthy family-contacts of pneumonia patients are throat-carriers of the infecting type of pneumococcus (Smillie, 1933; Finland and Tilghman, 1936).

Pneumococci of one type or another are carried in the throat by a substantial proportion of healthy persons not known to have had recent contact with pneumonia patients: reported pneumococcus throat-carrier rates include 34.8% in America (Buerger, 1905), 0% in Alabama and 30% to 70% in Labrador (Burky and Smillie, 1929), 6% in Puerto Rico (Pomales, 1929), 0% to 67%, mainly 20% to 40%, of 100 persons in Manchester during 3 years (Report, 1930), 11% and 13% in St. John, Virgin Isles (Milam and Smillie, 1931), 35% to 80% among children in Heidelberg during a year (Gundel and Linden, 1931), 20% to 80% of 50 infants in a New York institution during 9 months (Kneeland and Dawes, 1932), 20% to 59% of 15 students in Toronto during 7 months (Brown and Anderson, 1932), 7% among Eskimoes in the remote north (Wells and Heinbecker, 1932), 14% in an isolated Arctic community (Paul and Freeze, 1933), 6% and 1% in Puerto Rico (Lebrón, 1936) and 0% to 68%, mainly /

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mainly 20% to 40% among various groups of adults and children in London and South-East England during the years 1930 to 1937 (Report, 1939).

Finland (1942) has summarised American, British and German observations of the occurrence of the different serological types of pneumococcus in 12,049 adult cases of pneumonia, 2506 child cases of pneumonia and 3543 healthy carriers; every recognised type of pneumococcus has produced pneumonia on some occasions and every type has been found in healthy carriers on some occasions; adult pneumonia was due most frequently to types 1, 2, 3, 7 and 8 (respectively, 21.4%, 7.4%, 12.3%, 9.3% and 8.2% of cases), child pneumonia was due most frequently to types 14, 1, 6-26 and 19 (respectively, 17.3%, 15.3%, 12.3% and 7.5% of cases), and healthy carriers were infected most frequently with types 3, 6-26, 18 and 4 (respectively, 14.9%, 10.4%, 7.9% and 6.6% of carriers; type 1 in 1.2% and type 2 in 1.0%). The type distribution in bronchopneumonia patients of all ages was similar to that in healthy carriers (Finland,1937). These and other observations (e.g. Andrews, 1937) have suggested that the pneumococci are differentiated into "carrier types" of low invasiveness, such as types 3, 4, 18 and 19, which commonly are carried by healthy persons and commonly cause autogenous infections (e.g. secondary pneumonias and bronchopneumonias), but which show little tendency to spread to contacts or cause epidemic infections, and "infective types" of/

of high invasiveness, such as types 1, 2 and 14, which less commonly are carried by healthy persons, but which commonly cause exogenous infections (e.g. lobar pneumonia), readily spread from patients to contacts, and often cause epidemics with concurrence of numerous throat-carriers, cases of clinically-manifest upper respiratory tract infection and cases of lobar pneumonia. Gilman and Anderson (1938) describe an epidemic of this latter kind which was due to the type-1 pneumococcus and which occurred in a rural village community of 880 persons; there were 13 pneumonia cases and 22 other cases of pneumococcal ear infection, among which the proved carriage rate for type-1 pneumococcus was 34%; the type-1 pneumococcus was found to be carried in the throat by 12% of 82 family contacts of cases of pneumococcus disease and by only 1.4% of 138 non-contacts; most of these carriers rid themselves of the pneumococcus within 2 weeks, but some remained infected for 3 months.

The pneumococcus is carried fairly commonly in the mouth by healthy persons. Buerger (1905) found pneumococci in 50% of 78 normal mouths. Stillman (1917) found pneumococci in the saliva of 39% of 297 normal persons (type-1 in 0.33%, and type-2 in 0%); he found type-1 pneumococci in the saliva of 15% of 107 contacts of type-1 disease and found type-2 pneumococci in 6% of 77 contacts of type-2 disease. Pneumococci were found in 26% of mouth-secretion samples from healthy persons in Manchester who showed

a /

a nasopharyngeal-carrier rate of 50% (Report, 1930).

Pneumococci are carried in the nose less commonly than in the throat. Pneumococci were found in 17% of nasal swabs from healthy persons in Manchester who showed a nasopharyngeal-carrier rate of 41% (Report, 1930). Among healthy adults and children in London and South-East England, the nasal-carrier rate was mainly between 5% and 10%, about a quarter of the nasopharyngeal-carrier rate (e.g. in one case, 8% as compared with 30.8%) (Report, 1939).

Carriage of N.meningitidis.

In the case of the meningococcus, reports of nasopharyngeal-carrier rates include: 10% rising to 70% in an army barracks during an epidemic (Glover, 1918 a), 3% to 38% of 4000 non-contact soldiers in the London area (Glover, 1918 b), 6% to 34% among healthy "non-contacts" in London (Eastwood, 1918), 10% among 400 normal persons in East England (Ponder, 1918), 2.4% of 2455 swabs from healthy persons in Manchester (Report, 1930), 50%, mainly as transient carriers, continuously for a year among the inmates of a naval hospital (Dudley and Brennan, 1934), 42% of 24 healthy persons in America, some of whom carried the meningococcus for over 2 years, and 2% in an American labour camp (Rake, 1934), and 0% to 25.5%, mainly 10% to 20%, in groups of healthy persons in South-East England (Report, 1939).

The meningococcus is carried in the nose less commonly than in the throat; among persons in South- /

South-East England showing a nasopharyngeal-carrier rate of 12.3%, the nasal-carrier rate was 1.3% (Report, 1939).

Carriage of H.influenzae.

The influenza bacillus is carried in the throat by a large proportion of healthy persons. Bloomfield (1922 c) reviewed many early observations of the throat-carriage rate, which ranged from 10% to 90%. Further throat-carriage rates reported include: less than 20% in Alabama and Labrador (Burky and Smillie, 1929), 7% in Puerto Rico (Pomales, 1929), 31.7% to 80%, mainly 50% to 60%, in Manchester (Report, 1930), 47% in New York (Dochez, Shibley and Mills, 1930), 11% to 13% in St. John, Virgin Isles (Milam and Smillie, 1931), 13% among polar Eskimoes (Wells and Dixon, 1932), 16% in Spitzbergen (Paul and Freeze, 1933), 8% in Puerto Rico (Lebrón, 1936), and 25% to 84%, mainly 50% to 80%, in South-East England (Report, 1939). In the last mentioned investigation (Report, 1939), it was noted that only a small proportion of the "carried" H.influenzae strains belonged to the "smooth" type of Pittman, which is highly virulent for man; in an orphan's home where the total H.influenzae carrier rates were 78% for the throat and 54% for the nose, the corresponding rates for "smooth" strains were 8% and 10%; in a public school having a total throat-carrier rate ranging from 31% to 81%, the throat-carrier rate for "smooth" strains ranged between 0% and 19%.

The /

The influenza bacillus commonly is carried in the mouth by healthy persons. Haemophilic bacilli were found in 25% of mouth-secretion specimens from healthy persons in Manchester who showed a nasopharyngeal-carrier rate of 51% (Report, 1930). Fleming and MacLean (1930) found haemophilic bacilli on the gums of all of 30 persons, of whom 29 bore the bacilli on the tonsils and 26 in the post-nasal space.

The influenza bacillus is carried in the nose less commonly than in the throat. Haemophilic bacilli were found on 12% of nasal swabs from healthy persons in Manchester who showed a nasopharyngeal-carrier rate of 59% (Report, 1930). Dochez, Shibley and Mills (1930) reported an H.influenzae nasal-carriage rate of 0% among persons in New York having a nasopharyngeal-carrier rate of 47%. H.influenzae was found to be carried in the nose by 0% to 13%, on average 7.2%, of a group of healthy adults in London, and by 54% of the inmates of an orphans' home (Report, 1939).

Carriage of Staph.aureus.

The principal habitat of Staph.aureus is the nose of healthy persons; the staphylococci are found mainly on the skin lining the anterior nares; the mucous membrane lining the nasal cavity usually bears only a few staphylococci, ones which have passed back from the nostrils. The proportion of healthy persons carrying Staph.aureus in the nose has been reported as ranging from 20% to 80%, usually being about 50% (Hallman, /

(Hallman, 1937; McFarlan, 1938; Bartley, 1941). Devenish and Miles (1939) found 18 out of 27 attendants in a surgical theatre to be nasal carriers. Allison and Hobbs (1947) found that 72 (i.e. 70%) of 103 members of the staff of a maternity hospital carried Staph.pyogenes (aureus) in the nose at the time of an epidemic of staphylococcal infections among the mothers and babies; 34 staff carriers (33% of the 103 persons) harboured the serological type of staphylococcus which was causing the epidemic infections. Miles, Williams and Clayton-Cooper (1944) found that large numbers of Staph.aureus cells are present in the anterior nares of carriers and that the carrier state is of relatively long duration.

The throat is a much less common site of carriage of Staph.aureus. Bloomfield (1921 c) examined 8 healthy persons and found one (i.e. 13%) to be a persistent throat-carrier, yielding large numbers of Staph.aureus from both tonsils on all of 5 occasions, but none on any occasion from the nose or tongue. Campbell (1948) found that coagulase-positive staphylococci were carried in the throat by 4.4% of 158 healthy persons, by 11.7% of 469 patients with pharyngitis and by 53% of 66 patients with glandular fever.

Carriage of K.pneumoniae.

Bloomfield (1921 a) found that Friedlander's bacillus was carried in the throat by 5 (i.e. 5.8%) of 85 normal persons; carriage was persistent, the bacillus /

bacillus being found at almost all of many swabbings during a 1-month to 4-month period in all of four carriers so examined. Gundel and Schwarz (1932) found Friedlander's bacillus in 18% of 426 throat-swabs from 51 mothers. Among healthy adults and children in London and South-East England, the throat-carrier rate for Friedlander's bacillus was found to vary mainly between 1% and 8%; carriage was persistent (Report, 1939).

Sources Outside the Respiratory Tract.

Pathogenic bacteria such as Strept.pyogenes, Staph.aureus and M.tuberculosis frequently are found on the skin of persons who are harbouring these bacteria in the respiratory tract; the skin is acting merely as a temporary reservoir for germs disseminated from the respiratory tract. Only in the case of Staph.aureus is skin contamination perhaps sometimes due to colonisation, with the skin acting as a true source of infection. Burtenshaw (1942, 1945) has shown that the staphylococci are resistant to the unsaturated fatty-acids of the skin secretion which are bactericidal towards Strept.pyogenes, C.diphtheriae and other respiratory-tract bacteria. Staph.aureus skin-carrier rates of 5% to 24% have been recorded for healthy persons, but in most cases the staphylococci on the skin probably were "transients" derived from the nose; the majority of skin-carriers have been found to carry the same serological- or bacteriophage-type of staphylococcus in the nose as on the skin (Gillespie, Devenish and Cowan, /

Cowan, 1939; Miles, Williams and Clayton-Cooper, 1944): skin carriage, on the hand, was observed in 28 of 72 nasal carriers, but in none of 31 other persons who were not nasal carriers (Allison and Hobbs, 1947). However, Devenish and Miles (1939) showed that some persons, about 5% of healthy persons, carry Staph.aureus so deeply in the skin and so persistently that the coccus appears to be resident and multiplying in the skin (see also Gillespie, Devenish and Cowan, 1939; Vierthaler, 1940).

Staph.aureus has been found to be present commonly in the breast and milk of healthy mothers: in 1 out of 48 mothers (Cohn and Neumann, 1891), in 79 out of 137 mothers (Kostlin, 1897), and in 80 (i.e. 92%) of 87 nursing mothers but in only 6 (i.e. 21.4%) of 28 mothers who were lactating but not feeding their babies (Duncan and Walker, 1942). The observations made by Duncan and Walker in maternity hospitals suggested that the mother's breast was not the source of infection for the baby, but that it usually was infected from the baby with a strain acquired by the baby in the nursery. The number of Staph.aureus in freshly drawn infected milk is very large.

Infected sores, burns and wounds may act as an abundant source of Staph.aureus and Strept.pyogenes. Bourdillon and Colebrook (1946) have demonstrated that the air becomes contaminated with clouds of airborne bacteria during changing of the bandages on infected burns; contamination of the air both with Staph.aureus /

Staph.aureus and with Strept.pyogenes was observed in the dressing station. Colebrook and Ross (1947) observed contamination of the air of a dressing station with Strept.pyogenes which were traced by the serological typing method to having originated from a small scabbed-over lesion on the elbow of a surgeon, and, in another instance, to the bandages of a burn.

Animal Sources of Bacteria Pathogenic for the Human Respiratory Tract.

Domestic animals, including dogs, cows, hogs and sheep, have been found commonly to carry beta-haemolytic streptococci in their throat and tonsils, but the strains carried rarely have belonged to group A, or have corresponded with Strept.pyogenes, the streptococcus which is pathogenic for man (Pilot, Buck and Davis, 1936: Pilot, Buck, Davis and Eastman, 1936). Normal Rhesus monkeys have been found commonly to carry in the throat streptococci of group A; Seegal, Heller and Jablononowitz (1936) found group-A streptococci in 19 out of 49, group-C streptococci in 4 out of 49, group-G streptococci in 5 out of 49, and typable pneumococci in 33 out of 49 (including types 3, 4, 5, 7, 8, 19 and 32).

Cows' milk is an important source of M.tuberculosis, Strept.pyogenes, C.diphtheriae and Staph.aureus, quite apart from its frequent role as a vehicle of pathogenic bacteria introduced from human carriers. MacDonald (1946) found coagulase-positive Staph.aureus in more than 50% of 280 samples of accredited /

accredited cow's milk in Norfolk; the majority of strains were found to belong to one phage type, 42D, which was common in cases of bovine mastitis.

Dean and Todd (1902) described a small outbreak of milk-borne diphtheria in which the source of infection was two cows having papules and scabbed ulcers on the udder and teats; these sores, and also the milk of both cows, was demonstrated to contain virulent diphtheria bacilli.

Henningsen and Ernst (1938) described an epidemic of septic sore throat due to Strept.pyogenes (group A), which affected 100 persons out of a village of 750 inhabitants; the outbreak was traced to the milk supplied from a certain farm where one cow was found to be showing signs of subacute mastitis and to be discharging group-A streptococci in its milk. Subsequently, Bendixen and Minett (1938) studied this cow and found that the group-A streptococci continued during the whole of 13 months to be discharged in the milk from the left front quarter, which supplied ½% tc 15% of the total milk yield, in numbers varying from 150 to 820,000 per ml..

Watson (1937) reported an epidemic of infections due to Strept.pyogenes (group A, type 2), which affected persons from 54% of 380 families supplied with milk from a certain farm. Subsequently, a cow on this farm, which had an indurated right front teat, was studied by Bendixen and Minett (1938) and found to be discharging group-A, type-2, streptococci from its right and left front teats, in numbers which, during /

during the course of 6 weeks, varied from 280 to 10,000,000 per ml..

Douglas, Smith, Sutherland and Watson (1941) described an epidemic of 214 cases of scarlet fever due to Strept.pyogenes (group A, type 3); 189 of the 214 cases had been supplied with milk from a certain herd; an apparently healthy cow in this herd was found to be discharging group-A, type-3 streptococci in the milk from a quarter which seemed normal at the time and only later became indurated.

Carriage of Viruses.

Viruses causing such diseases as influenza and the common cold, frequently have been demonstrated by filtrate-inoculation experiments to be present in the secretions of the upper respiratory tract of patients; the specimens examined have been rinses of the mouth and throat, or nose and nasopharynx, and information is not available as to the precise localisation of the virus in such patients, for instance as to whether it occurs in the anterior mouth as well as in the throat.

Very little is known about whether viruses occur in "healthy carriers". Eaton and van Herick (1944) and Horsfall and Curnen (1946) have shown that the mouse pneumonia virus may be harboured by certain animals of other species, and be transmitted from one to another, without causing apparent clinical infection; in fact, these animals are "healthy carriers" of the virus. Hare and Mackenzie (1946) have reviewed many published observations of common cold, influenza or measles breaking out in isolated /

isolated communities after the arrival of travellers who were themselves free from clinically-manifest infection; although very suggestive, these observations can not be taken as conclusive proof of the occurrence of true "virus carriers", since it is possible that the virus was transported, not in the respiratory tract of the traveller, but in some reservoir such as his clothing or luggage, which had been contaminated by a patient at a place visited previously. If such "virus carriers" do exist, then, according to Hare and Mackenzie, they can not be very common, since the majority of common cold, influenza and measles cases appear to be secondary to known cases, and isolated communities often escape infections which spread universally elsewhere. Furtherevidence suggesting that viruses may be carried in the throat by healthy persons, is provided by the studies of Broadhurst and her colleagues (1936, 1938, 1943 a,b) on the occurrence of cytoplasmic inclusion bodies in the throats of "normal" persons. Searching for "carriers" of the influenza virus, McKee and Hale (1949) examined nose and throat washings from 50 healthy persons and tracheal specimens from 100 persons at necropsy; virus was recovered from only 1 of the 150 cases, a necropsy case who possibly had been suffering active influenza.

(5) Dissemination of Bacteria from Respiratory Tract in "Entire Discharges" of Saliva, Throat Secretion, Nasal Secretion and Sputum.

The respiratory-tract secretions may be distributed in two ways: either as "entire discharges" or as "droplet-spray". The term, "entire discharges", is used to distinguish all forms of distribution other than by spraying. The "entire discharges" include: expectoration from the mouth of saliva, sputum and throat "hawkings", blowing from the nose of nasal exudate and removal from the nose and mouth of small quantities of these secretions on the fingers, on eating utensils and in kissing.

The rôle of discharged sputum and saliva in the transmission of tuberculosis will be considered separately in a later section. In relation to tuberculosis, Neild and Dunkley (1909) drew attention to the many ways in which persons commonly distribute their saliva: in licking envelopes and threads, in licking the fingers for turning the leaves of a book and for other purposes, and in sucking pencils.

Chapin (1912) regarded the distribution of saliva by the fingers as the main mechanism of contagion; he wrote: "Probably the chief vehicle for the conveyance of nasal and oral secretion from one to another is the fingers. If one takes the trouble to watch for a short time his neighbours, or even himself, unless he has been particularly trained in such /

such matters, he will be surprised to note the number of times that the fingers go to the mouth and the nose. Not only is the saliva made use of for a great variety of purposes, and numberless articles are for one reason or another placed in the mouth, but for no reason whatever, and all unconsciously, the fingers are with great frequency raised to the lips or the nose. ---- All successful commerce is reciprocal, and in this universal trade in human saliva the fingers not only bring foreign secretions to the mouth of their owner, but there changing them for his own, distribute the latter to everything that the hand touches. ---- The cook spreads his saliva on the muffins and rolls, the waitress infects the glasses and spoons, the moistened fingers of the pedlar arrange his fruit, the thumb of the milkman is in his measure, the reader moistens the pages of his book, the conductor his transfer tickets, the "lady" the fingers of her glove. Everyone is busily engaged in this distribution of saliva, so that the end of each day finds this secreton freely distributed on the doors, window-sills, furniture and playthings in the home, on the straps of trolley-cars, the rails and counter and desk of shops and public buildings, and indeed upon everything that the hands of man touch."

Undoubtedly, <u>fingering the mouth</u> and <u>picking the nose</u> are two exceedingly common habits; <u>spitting</u> and <u>blowing the nose</u> are much less common, and it is questionable /

questionable whether they are as important as the former habits, in spite of the much larger volume of secretion which they distribute on each occasion.

The greater importance of expectorated "hawkings" from the throat, as compared with saliva distributed by contact, was suggested by the observations of Bloomfield and Felty (1924) who found numerous haemolytic streptococci in the expectorated "hawkings" of two patients with acute tonsillitis, but none in the anterior-mouth secretions removed, before the "hawking", on a culture plate which was kissed and on a spatula which was held in the mouth for several minutes.

On the other hand, a rather greater importance of the rôle of saliva appears probable in view of the finding by Hamburger (1944 b) that haemolytic streptococci were present, mainly in numbers between 1000 and 1,000,000 per ml., in 64.6% of 527 samples of expectorated saliva from 156 patients with scarlet fever or streptococcal tonsillitis-pharyngitis.

Hamburger and Green (1946) observed that blowing the nose is common among patients suffering from streptococcal upper respiratory disease and also among ambulatory carriers, and they found that many more haemolytic streptococci were expelled from the upper respiratory tract by blowing the nose than by either sneezing or coughing. The "pour-plate" method was used to count the viable haemolytic-streptococci which were expelled from carriers into a sterile /

sterile, 5-inch square, muslin handkerchief by 3 "nose-blows", by 3 coughs and by a sneeze; streptococcus counts were made on washings from the carrier's hands before and after the nose-blowing. "Nose-blowing" by carriers with strongly positive nose and throat cultures was found usually to expel large numbers of haemolytic streptococci into the handkerchief and on to the hand holding the handkerchief; the handkerchiefs acquired usually between 10,000 and 10,000,000 haemolytic streptococci (i.e. in 73% of cases), in some cases up to or exceeding 1,000,000,000, and on average 11,000,000; the hands acquired on average 450,000 haemolytic streptococci. Coughing by carriers with strongly positive nose and throat cultures expelled into the handkerchief between 0 and 47,000 haemolytic streptococci, on average 3700. Sneezing by carriers with strongly positive nose and throat cultures expelled into the handkerchiefs from 50 to 50,000,000 haemolytic streptococci, on average 3,600,000 (i.e. 3,000,000 into handkerchief over nose plus 600,000 into handkerchief over mouth); fewer than 10,000 streptococci were expelled by 56% of the sneezes. Hamburger and Green concluded that blowing the nose is the most important of the respiratory activities which result in the dissemination of haemolytic streptococci under epidemic conditions.

(6) Dissemination of Bacteria from Respiratory Tract in Droplet-Spray.

Koch (1884), having shown that artificially-sprayed tubercular sputum readily caused infection of animals, suggested that under natural conditions man might be infected by particles of sputum sprayed from the mouth of patients in coughing; however, Koch thought that this would not be a common means of infection because the cough-droplets would be too large to remain airborne for any length of time.

Flügge (1897) demonstrated that artificial atomisation of a bacterial suspension causes the air to become contaminated with bacteria-carrying droplets which remain airborne for a considerable time. He arranged that a jet of air having a velocity of 4 meters per second, or greater, struck at an angle of 45 degrees on the surface of a suspension of B.prodigiosus: on examining the exhaust air, he found that this bore in suspension droplets infected with B.prodigiosus. Flügge suggested that the air of dwellings might become infected by the spraying of saliva in speaking, coughing and sneezing. On the basis of observations by his pupils, particularly those made by Laschtschenko, Flügge (1899, 1901) suggested that the cough-spray of patients is the main means of spread of pulmonary tuberculosis. He concluded from the various findings that the respiratory droplets are fairly coarse and settle quickly, and that although some may remain suspended in /

in the air for as much as several hours, these are few in number and hygienically negligible.

Expulsion of Droplets by Different Expiratory Activities.

The majority of investigators have found that bacteria are not readily detached from moist surfaces, such as those of the respiratory tract, and that bacteria are not expelled in the air which is quietly expired in normal breathing (e.g. Tyndall, see Chapin, 1912; Carnelley, Haldane and Anderson, 1887). Koelzer (1903) found tubercle bacilli on 1 out of 15 occasions in a dish breathed at for 7 to 15 minutes by a consumptive; he concluded that tubercle bacilli are sometimes, though only rarely, expelled by the normal breathing of consumptives, atomisation perhaps occurring within the tuberculous lung. Meleney (1927) opined that bacteria are discharged from the nose during normal breathing, either in droplets or in dry particles blown off the hairs in the nostrils. Hirshfeld and Laube (1941) found that many bacteria were given off into the air of a small cabinet into which was inserted the head of a person who was breathing quietly. Hamburger and Green (1946) found that even forcible audible mouth-breathing by carriers did not ordinarily expel many haemolytic streptococci; in experiments with 16 carriers, the latter breathed 20 times at a culture plate held 2 inches from the mouth; 62% of plates did /

did not collect any haemolytic streptococci and 17 colonies of the streptococcus was the greatest number collected on any plate. Audible breathing through the nose by 7 subjects with strongly positive nose cultures, resulted in the recovery of but 1 colony of the haemolytic streptococcus on 1 of the 7 plates. Bourdillon, Lidwell and Lovelock (1948) pointed out that bacteria found to be distributed during normal breathing, as by Hirshfeld and Laube (1941), may not have come from the respiratory tract, but instead have been dislodged by minor movements from the subject's hair, skin or clothing, particularly from the head and neck. Bourdillon and his colleagues used a slit sampler to examine the air of a small chamber in which sat the test subject breathing quietly; only one or a few bacteria-carrying particles were found per 35 cu.ft. of the chamber air when the subject was dressed in a clean oiled gown and had his hair, face and neck oiled, but larger numbers were found in earlier tests when the hair, face and neck were not oiled; these authors believe that no bacteria are emitted in the quiet breathing of a healthy person.

Expulsion of droplet-spray in <u>speaking</u>, <u>coughing</u> and <u>sneezing</u>, was first studied by Laschtschenko (1899) in experiments with a subject whose mouth had been artificially infected with a small quantity of a heavy suspension of <u>B.prodigiosus</u>. A dozen agar culture plates were exposed at mouth height in various /

various parts of the test chamber, of 3.2, 50 or 90 cubic meters, during the expiratory activity and for an hour subsequently. As B.prodigiosus normally is not found in the air, the number of characteristic red colonies developing on the plates was assumed to represent the number of mouth-secretion droplets falling on these.

Soft speaking for 1 hour gave only a few colonies, 1 to 8, on a few of the plates in the 3.2-cu.m chamber, and not any on the plates in the 90-cu.m. chamber. Loud speaking for 1 hour gave several colonies, mainly between 1 and 10, on a majority of the plates exposed at 40 to 600 cm. from the speaker in the 50-cu.m. and 90-cu.m. chambers; a plate exposed at 600 cm. yielded 1 colony. Coughing loudly 10 times gave innumerable colonies on the nearest plate and several, totalling 114, on each of the other 11 plates exposed at 40 to 185 cm. distance in the 3.2-cu.m. chamber.

Sneezing 5 to 8 times under the influence of snuff gave many colonies on all plates exposed at 100 to 600 cm. distance in the 50-cu.m. chamber; the 12 plates exposed yielded a total of 959 colonies. Thus, sneezing was the most prolific distributor of mouth-secretion, and speaking, especially soft speaking, the least prolific. Such tests did not distinguish between droplets which remained suspended in the air for a time before settling on to the plates and droplets which were projected directly through /

through the air from the mouth to the plates, although it may be assumed in the light of present-day knowledge that the "droplets" collected on plates at 6 meters from the speaker, could not have been projected that distance, but must have been carried in suspension by the air currents. Laschtschenko made a special experiment in the 3.2-cu.m. chamber to demonstrate conclusively that coughing produces infection of the air; air was withdrawn from the chamber through glass tubes and by culture it was shown that many B.prodigiosus were carried into the tubes; these bacilli must have been suspended in the air.

Hübener (1898) exposed 4 agar plates at about 50 cm. in front of and below the mouth of a subject who had infected his mouth with B.prodigiosus. In 11 tests of speaking for 10 minutes, the number of colonies on the 4 plates varied from 101 to 1507, averaging 458; two tests with 3 or 4 coughs gave 223 and 265 colonies; in two tests a single sneeze gave innumerable colonies.

Weismayr (1898), in similar tests, found B.prodigiosus on plates up to 4 meters in front of a coughing subject, but, when the air was undisturbed, not outside the zone directly in front of the mouth. When the air of the room was agitated, he found a few B. prodigiosus at 1 to 2 meters behind the subject, and also to one side of him; this indicated that some of the droplets had remained suspended in the air /

air for a time sufficient to allow transportation over this distance. In speaking experiments, B.prodigiosus was not found at further than 1 meter.

Koeniger (1900) made the most exhaustive of all studies of the dissemination of B.prodigiosus in mouth-spray. In 18 tests of speaking, usually for 15 minutes, B.prodigiosus was recovered on plates exposed in all parts of a 97-cu.m. or 440-cu.m. test room, at the extreme distance of 12 meters in front of the speaker, and also at one side of him and behind him. The average number of colonies per plate was 14.5 for sharp speech, 7.8 for loud speech, 2.1 for medium speech, 1.4 for soft speech and 5.3 for whispering. Most droplets were produced by the consonants "P", "T", "F" and "K". Koeniger made important observations on the duration of air-carriage of those droplets which remain suspended in the air. He never found B.prodigiosus in quiet air at later than 1 hour after the speaking had ceased, although when the air was agitated by opening and shutting of the door, he obtained positive results after 1½ hours. Taking as 100% the number of B.prodigiosus-droplets settling on the plates exposed during the speaking, he found only 38% after 10 minutes, 9.8% after 20 minutes, 5.5% after 30 minutes, 2.7% after 45 minutes, 0.7% after 60 minutes and 0.0% after 90 minutes. In similar experiments with B.mycoides, droplets containing this bacillus were not found settling from the air later than 10 minutes after the speaking.

Gordon /

Gordon (1904) suggested that studies of droplet-spray dissemination might be made by observation of certain streptococci which occur normally in saliva, instead of by observation of B.prodigiosus which must be introduced artificially. Examining the saliva of 25 healthy persons of from 2½ to 64 years of age, he found that Strept.brevis was invariably present and was much the most numerous of the salivary commensal bacteria, there being over 10,000,000 per ml. in all 25 samples and between 100,000,000 and 1,000,000,000 per ml. in 10 of the 25 samples. For the purpose of comparison, Gordon made both experiments with B.prodigiosus and experiments with Strept.brevis. He tested loud speaking, usually for about 1 hour, in a 1200-cu.ft. or a 28,000-cu.ft. room. A number of 3½-4 inch diameter culture-plates were exposed in different positions throughout the room. In 16 tests with B.prodigiosus it was found that droplets from the mouth were scattered through the air for distances of up to 40 feet in front of the speaker, and even to a point 12 feet behind him. In the tests without artificial contamination of the mouth, Strept.brevis was collected in plates containing broth and was identified by microscopical examination of this after incubation; Strept.brevis, like B.prodigiosus, was recovered at up to 40 feet in front of the speaker and at 12 feet behind him, being found in a total of 22 out of 30 plates exposed in 5 tests in the small room and in 11 out of 34 plates

and /

and 28 out of 40 plates in 2 tests in the large room. Gordon noted that the extreme distances of spread depended on carriage of suspended droplets by the air currents of the room.

Winslow and Robinson (1910) studied the distribution in droplet-spray of B.prodigiosus and of the lactose-fermenting Strept.salivarius. A subject whose mouth was infected with B.prodigiosus culture, undertook loud speaking for 15 minutes in a 178-cu.m. room or a 326-cu.m. room. Culture plates were exposed at different distances from the speaker, both during the speaking and during the subsequent 30 to 45 minutes. In all, 780 B.prodigiosus colonies were obtained on 325 plates, an average of 2.4 per plate per 48-minutes exposure. The average number of colonies per plate was 0.15 at 1 meter from the speaker, 2.22 at $1\frac{1}{2}$ meters, 3.96 at 2 meters, 5.73 at $2\frac{1}{2}$ meters, 4.16 at $3\frac{1}{2}$ meters, 3.08 at $4\frac{1}{2}$ meters, 1.85 at $5\frac{1}{2}$ meters, 2.28 at 7 meters and 1.35 at $7\frac{1}{2}$ meters. In the small room, which had a floor area of 480,000 sq.cm., a total of 774 B.prodigiosus colonies were collected on 254 plates having a surface area of 54.5 sq.cm. each. Assuming that all the bacteria above a plate settled on to that plate during the 45 to 60 minutes of exposure, the average number of B.prodigiosus-containing droplets sprayed into the room per 15 minutes of speaking must have been about 31,000. These colony counts on exposed plates did not distinguish the truly airborne droplets from the projectile /

projectile and immediately-settling droplets. Accordingly, Winslow and Robinson made special examinations of the air for the presence of suspended (airborne) infected particles, in most cases by admitting air to a previously evacuated sterile 2½-liter bottle having nutrient gelatine in the bottom, or by covering a Petri culture-dish with a 20-cm. high cylinder, and allowing sedimentation of the airborne particles during 30 minutes. In 16 of the 15-minute speaking experiments with B.prodigiosus, a total of 140 one-liter samples of air were taken just after the speaking, and B.prodigiosus was found in only 7 of these samples. In experiments with 10 to 20 minutes speaking by a subject whose mouth was not artificially infected, 74 one-liter samples were taken during the speaking; lactose-fermenting streptococci were not found in any of the samples. Winslow and Robinson concluded that the normal mouth bacteria are not disseminated as freely in droplet-spray as are the artificially-introduced B.prodigiosus, and that the amount of bacterial contamination of the air caused by droplet-spray is very slight.

Doust and Lyon (1918) exposed agar plates on a table in front of a subject whose mouth and throat had been rinsed with a suspension of B.prodigiosus, leaving them open during the expiratory activity and for 10 minutes subsequently; they found that speaking softly for 5 minutes sprayed a few B.prodigiosus- /

B.prodigiosus-containing droplets up to a distance of 2 feet from the subject, that speaking loudly for 5 minutes sprayed a greater number of such droplets up to a distance of 4 feet, and that coughing for 5 minutes sprayed many droplets up to a distance of 7 feet and a few droplets up to 10 feet.

Weaver (1919) counted the colonies of Strept.viridans developing on blood-agar plates which were exposed at 1 foot in front of the mouth of a normal subject. With the plates held so close to the mouth, the counts obtained should represent a major proportion of the total droplet output. The average colony counts were: 1 for talking 15 seconds, 1 for twice coughing with lips widely open, 2 for whistling 15 seconds, 4 for whispering faintly 15 seconds, 50 for twice blowing, 100 for once hawking, 100 for stuttering loudly 15 seconds, 200 for twice coughing with lips slightly parted, 300 for once sneezing and 670 for twice "forcing the lips slightly apart with a puff".

Bourdillon and Lidwell (1941) obtained 19,000 colonies on a 60-sq.in. serum-agar plate exposed at 3 feet in front of the mouth during a sneeze.

Mechanism of Airborne Infection with Droplet-Nuclei.

The early investigations, particularly those of Koeniger (1900) and of Winslow and Robinson (1910), afforded clear evidence that a proportion of the respiratory/

respiratory droplets remain suspended in the air for a considerable time, and thus that droplet-spray may produce true air infection. However, it was only by the investigations of Chaussé (1913 a,b), of Lange and Keschischian (1925) and of Wells (1934) that a proper understanding was obtained of the mechanism of air infection by droplet-spray and of the clear-cut distinction between, on the one hand, the large droplets which immediately fall to the ground and do not cause air infection, and, on the other hand, the small droplets which dry at once to solid residues, "droplet-nuclei", and as such remain suspended in the air causing air infection.

It was realised from the first that the duration of air carriage of droplets must depend upon their size. Buchner, Megele and Rapp (1899) found that artificial spraying of a culture-suspension yielded numerous bacteria-carrying droplets which were small enough to remain airborne while being transported by air currents to a considerable distance. Hutchison (1901) in one experiment found that after fine spraying of a B.prodigiosus culture, this bacillus was carried 55 meters along a corridor and up two flights of stairs.

Chaussé (1913 a) used the Richardson apparatus with a jet velocity of 150 meters per second to atomise an aqueous solution of methyl violet, spraying horizontally into a 13-cu.m. or 60-cu.m. room. The coloured droplets and particles were collected on sheets /

sheets of paper and glass-slides which were examined microscopically, and also into dishes of alcohol for colorimetric estimation. By examination of slides held close to the atomiser it was found that the spray consisted of droplets ranging in diameter from 2 to 2000 microns. By examination of slides exposed in various positions throughout the room, and at different times after atomisation, the subsequent course of the droplets was discovered. The large droplets, of 200 to 2000 microns, were projected to a distance of $1\frac{1}{2}$ to 2 meters from the atomiser, their momentum carrying them forward beyond the range of the air current issuing from the atomiser; all of these large droplets fell out of the air within 5 seconds after their formation. The medium droplets, of 15 to 200 microns, having less momentum, were projected only up to $1\frac{1}{4}$ meters; they all fell out of the air within 60 seconds after their formation. The small droplets, of 2 to 15 microns at collection, remained longest suspended in the air, being the only droplets remaining airborne for more than 60 seconds, and travelled the longest distances, passing to all parts of the room, even to as far as 10 meters from the atomiser. Chaussé pointed out that the time of suspension and the travelling range of these very small droplets are made especially great by their instantaneous desiccation; their mass, which originally is very small, is still further reduced by evaporation of their water content. Since the /

the minute dry droplet-residues can be carried by the least current of air and so be transported to considerable distances, they were recognised by Chaussé as possibly constituting an important vehicle of infection, one which was quite distinct from the large, rapidly-falling "droplets" of Flügge. Tests made by colorimetric estimation of the material settling at different times after atomisation, indicated that the proportion of the atomised material remaining suspended in the air was 8.3% after 1 minute, 5.5% after 5 minutes, 1.8% after 10 minutes, 0.93% after 20 minutes, 0.25% after 40 minutes, 0.1% after 60 minutes, 0.06% after 80 minutes and 0.03% after 100 minutes. The maximum time of suspension of droplet-residues was found to be 7 hours in experiments in which fluid containing tubercle bacilli was atomised into a room and air infection was observed by exposure of guinea pigs within the room at different times after atomisation; the guinea pigs exposed within 7 hours contracted inhalation tuberculosis.

Chaussé (1913 b) studied the passage of airborne dye-containing droplets and droplet-residues through glass tubes simulating the bronchial tract. The droplet-residues, of 2 to 15 microns in diameter, could be carried through a tube with a dozen 60-degree bends at a speed of 250 cm. per second, that is about the speed of normally inspired air. The large droplets, of over 50 microns in diameter, were deposited /

deposited on the bottom of the tube by gravity and could not be drawn through it.

These findings of Chaussé (1913 a,b) were confirmed in similar experiments by Lange and Keschischian (1925). An aqueous solution of eosin, or some sputum homogenised by shaking with glass beads, was artificially sprayed into a closed glass chamber, giving droplets of 2 to 1000 microns in diameter. At various times after spraying, slides were exposed to collect the droplets. Droplets larger than 200 microns all fell to the floor within a few seconds and droplets larger than 20 microns all fell to the floor within a few minutes; droplets smaller than 20 microns remained suspended in the air for a longer time, occasionally even for 2 or 3 hours.

Lange and Keschischian also studied the passage of airborne dye-containing droplets of different sizes through glass tubes of different widths and shapes. Only those "droplets" which were smaller than about 20 microns in diameter were capable of being drawn through a spiral tube with an internal diameter of 6 mm. and with 3 turns of 5 cm. diameter; the diameter of the largest "droplet" passing through this tube in an air current of 30 cm. per second was 24 microns, at 100 cm.p.sec. 20 microns, at 150 cm.p.sec. 16 microns, and at 400 cm.p.sec. 12 microns; the air currents of 150 and 400 cm. per second were produced by natural breathing. Many droplets of 20 to 100 microns passed at all speeds through a 10-mm. diameter /

diameter tube having one right-angle bend. Droplets larger than 100 microns in diameter passed in numbers through a 10-mm. diameter straight tube only.

By calculation from basic physical data, Wells (1934) reached the same conclusions as did Chaussé, and Lange and Keschischian by experimental observations. Wells did not acknowledge any acquaintance with the work of these earlier investigators. He gave the name of "droplet-nuclei" to the dry droplet-residues. Wells pointed out that according to Newton's law the falling time from mouth to ground would be less than 1 second if the droplets were not resisted by the air. In determining the time of fall through the height of a man, air resistance is a negligible factor only in the case of droplets larger than 0.1 mm. in diameter. As droplets decrease in diameter, the volume and weight, and thus gravitational pull, decrease more rapidly than the surface area, which latter determines the resistance to fall through air. Particles smaller than 0.1 mm. in diameter soon reach a falling velocity which remains constant and which, according to Stokes' law, is proportional to surface area, that is square of diameter. By calculation, Wells showed that the time of falling two meters in water-saturated air is 0.6 seconds for droplets of 1.0-mm. diameter or larger, 6.0 seconds for 0.1-mm. droplets, 10 minutes for 0.01-mm. droplets and 16.6 hours for 0.001-mm. droplets. In unsaturated air, evaporation of /

of their water content reduces the volume of the droplets and this shrinkage becomes more rapid as the droplet decreases in size. At constant temperature and humidity, evaporation proceeds so that the rate of change of surface area is constant; thus, the "life" of droplets in unsaturated air is proportional to the square of their diameter. From data given by Whytlaw-Gray and Patterson (1932) it was calculated by Wells that the evaporation time of water droplets in unsaturated air at 18 deg.C. is 11 minutes for droplets of 2-mm. diameter, 2.75 minutes for 1-mm. droplets, 41 seconds for 0.5-mm. droplets, 6.6 seconds for 0.2-mm. droplets, 1.7 seconds for 0.1-mm. droplets and 0.4 seconds for 0.05-mm. droplets. Thus, minute droplets settle slowly and evaporate almost immediately, whereas large droplets settle rapidly and reach the ground without appreciable loss by evaporation. The distance a droplet will fall before complete evaporation, is mainly dependent on its size, being proportional to the fourth power of the diameter, and only to a lesser extent is influenced by other factors, being proportional to the first power of the temperature and the first power of the humidity. Wells calculated that the maximum size of droplet which would evaporate completely in falling 2 meters, would be 0.172 mm. in air at 0% relative humidity, 0.145 mm. at 50% R.H., 0.128 mm. at 70% R.H. and 0.097 mm. at 90% R.H. Droplets derived from body secretions /

secretions contain certain dissolved substances and suspended bodies, including bacteria, so that on complete evaporation of their water content they leave solid residues, or "droplet-nuclei". All those respiratory droplets which originally are smaller than 0.1 mm. in diameter will evaporate before falling to the ground and will thus form droplet-nuclei. The size of the droplet-nucleus depends on the amount of solid matter in the droplet, and as this amount is very small in body fluids the droplet-nucleus will be much smaller than its parent droplet, and, correspondingly, will fall through the air at a much slower rate, will remain airborne for a much longer time and will be capable of transportation over long distances by slight air currents. Wells concluded that the transmission of infection through air may take one of two forms depending on the size of the infected droplet: namely, droplet infection proper, by the droplets larger than 0.1 mm. in diameter, which are rapidly and within a short distance removed from the air by gravity (before they can dry), and airborne infection, by droplet-nuclei derived from droplets smaller than 0.1 mm. in diameter, which remain suspended in the air for long periods of time and are transported over considerable distances.

Wells and Stone (1934) studied the duration of artificially-produced droplet-nucleus air infection. They used a compressed air atomiser to spray into a 200-cu.ft. tank various bacteria suspended in water or /

or broth, atomising 25 ml. into about 1,000,000 droplets per ml.. At intervals after the spraying, samples of air were withdrawn from the tank and examined with the Wells air-centrifuge. The sporing bacterium, B.subtilis, disappeared from the air at the rate of about 90% per day, being found in the air for 5 to 7 days; as the tank was unventilated and the spores unlikely to die, this disappearance may be attributed entirely to sedimentation. Other bacteria disappeared from the air more rapidly, presumably because of a greater death rate; living Staph.aureus, C.diphtheriae, Dip.pneumoniae, Strept.viridans and Strept.pyogenes were found in the air for 2 days, B.prodigiosus, E.coli, Ps.pyocyanea, Salm.typhosa and Sh.dysenteriae (Hiss B) for 2 to 8 hours, and H.influenzae for only $\frac{3}{4}$ hour.

Phelps and Buchbinder (1941), using both exposure of "settling plates" and a Wells air-centrifuge, studied the duration and course of air infection by Strept.viridans-containing droplet-nuclei which were put into the air of a closed and unventilated 445-cu.ft. chamber by artificial atomisation of a broth culture at an air-jet velocity of 360 meters per second. "Settling plate" observations showed that, on average, of the ultimately settleable infected nuclei, 78% were still airborne after 1 hour, 51% after 3 hours and 16% after 8 hours; parallel air-centrifuge observations gave similar values. The number of infected nuclei /

nuclei deposited, decreased geometrically with time. The nuclei remaining airborne must therefore have been kept in uniform distribution throughout the chamber by the inevitable minor convection currents. Uniform distribution of the nuclei was demonstrated directly in later experiments described by Phelps (1942); "settling plates" simultaneously exposed at different levels in the chamber collected similar numbers of nuclei in the same periods of time. The composition of the spray was investigated by atomising a strong solution in broth of the dye "uranine", instead of a Strept.viridans culture, and estimating the collected material colorimetrically. The uranine settling-rate data enabled formulation of a size-distribution for the droplet-nuclei, indicating that 95% by weight of the airborne material consisted of nuclei ranging from 0.34 to 5.4 microns in diameter (Phelps, 1942). On the assumption that the Strept.viridans-carrying nuclei would correspond to the half of the size-distribution larger than 1.34 microns in diameter, and with allowance for the normal streptococcus death rate, the streptococcus settling-rate data were in good agreement with the uranine date (Phelps and Buchbinder, 1941; Phelps, 1942). Phelps and Buchbinder state that the settling velocity is 7.7 inches per hour for a 1.36-micron nucleus, 25 inches per hour for a 2.4-micron nucleus and 125 inches per hour for a 17.0-micron nucleus.

Production /

Production of Droplet-nuclei by Expiratory Activities.

Although it has thus been shown that artificial atomisation of cultures produces many infected droplets small enough to remain airborne as droplet-nuclei, it does not necessarily follow that natural expiratory activities, such as speaking, coughing and sneezing, will also do this. The earliest direct demonstrations of air infection being produced by these expiratory activities were made without knowledge that the airborne bacteria were carried by dry droplet-nuclei rather than by liquid droplets: Laschtschenko (1899) and Winslow and Robinson (1910) found a few B.prodigiosus-containing "droplets" in suspension in air sampled after coughing or speaking; Weismayr (1898), Koeniger (1900) and Gordon (1904) found infected "droplets" settling on culture plates exposed behind the person coughing or speaking; and Koeniger (1900) found many infected "droplets" still settling out of the air at various times up to 1 hour after production by speaking.

Wells (1935) found that a sneeze produced over 20,000 bacteria-carrying droplet-nuclei. Wells and Wells (1936) used the air-centrifuge to examine the air of a room before, during and after 50 sneezes given by a group of persons using sneezing powder; before the sneezing the air contained only 2 bacteria-carrying particles per cu.ft., during the sneezing 309 per cu.ft. and after the sneezing 144 per cu.ft; the droplet-nuclei carried mouth commensal /

commensal bacteria and about two-thirds of the colonies were alpha haemolytic, presumably Strept. viridans.

Hare (1940 a) failed to demonstrate the production of bacteria-carrying droplet-nuclei by speaking; with the air-centrifuge he sampled air drawn through a funnel held 1 foot directly in front of the mouth of a person during 5 minutes of speaking and he recovered alpha streptococci not much more frequently than in control samples taken before the speaking.

Bourdillon, Lidwell and Lovelock (1942), using the slit sampler to examine the air of a small room, found that a snuff-induced sneeze on average gave rise to about 100,000 bacteria-carrying droplets which were small enough to remain airborne as droplet-nuclei for at least one minute; subsequent sampling at intervals revealed that the number of bacteria-carrying nuclei decreased geometrically with the elapse of time after sneezing, on average 4% remaining suspended in the air for as long as 30 minutes.

Du Buy, Arnold and Olson (1947) counted Lactobacillus acidophilus as an indicator of respiratory-tract origin, this bacterium normally being present in saliva in numbers ranging from 0 to 1,000,000 per ml.; except on 1 occasion out of 17, lactobacilli were not recovered in 20 cu.ft. samples of air taken into a Folin bubbler from a point 6 inches /

inches in front of the mouth of a person speaking for 20 minutes; only a few lactobacilli were collected on "settling plates" exposed at 8 inches below the mouth. The negative results of these investigators may be attributed to unsuitability of the lactobacillus as an indicator, due to, among other things, its presence in the mouth secretions in insufficient numbers.

Bourdillon, Lidwell and Lovelock (1948) used the slit sampler to count the bacteria-carrying droplet-nuclei expelled during speaking for 2 minutes into the air of a small, 15 cu.ft., chamber; the average number of such nuclei put into the air by each of three subjects was 1540, 420 and 45.

Mechanism of Formation, Sites of Origin, and Sizes of Respiratory-Tract Secretion Droplets.

In a review, Jennison (1942) points out that the mechanism of droplet formation from respiratory-tract secretions, in sneezing, coughing and speaking, is the same as that described by Castleman (1931) for the"atomisation" of other liquids by an air stream. A portion of the liquid is caught up by the air stream, and, being anchored at one end, is drawn out into a fine filament. This filament is quickly cut off by the rapid growth of a dent in its surface, and the detached mass, being quite small, swiftly draws itself up into a spherical droplet. The higher the air speed, the finer will be the filaments, the shorter /

shorter their lives, and the smaller and more numerous the droplets. High-speed photographs by Jennison and Edgerton (1940) and Turner, Jennison and Edgerton (1941), and by Bourdillon and Lidwell (1941), show clearly the presence in sneeze-spray of some unusually large filaments of secretion which are in the process of breaking up into a chain of droplets.

Air velocities high enough for spray formation are likely to be produced when the breath is forced out through some part of the respiratory passages which has been greatly narrowed. The site of narrowing, and thus the site of origin of the droplets, may be at the front of the mouth when this is almost closed by approximation of the tongue, teeth and lips, in the throat when this is nearly closed by approximation of the tongue, tonsils and and soft palate, in the glottis when this is nearly closed by the vocal folds, in a bronchus when it is obstructed by secretion, in the nasal cavity when this is obstructed by secretion, or in the anterior nares which are the narrowest parts of the normal nasal passages.

Sauter (1928), by photometric measurement of the droplets produced on mechanical atomisation of water, found that the mean droplet diameter decreased as the air-stream velocity increased, until a lower limit of 12 microns diameter was reached at air speeds of 100 meters per second and higher; there was much variation in the size of the droplets formed /

formed at any one air speed. The extent of this size variation was studied by Phelps (1942) in the case of a solution of uranine in broth which was atomised mechanically at an air speed of 360 meters per second; the variation in size, as calculated from the observed droplet-nucleus settling rates, was about 4-fold within the central 67% of the material atomised and about 16-fold within the central 95%. The applicability to respiratory secretion droplets of these measurements made in the case of artificial sprays, depends upon the similarity of the conditions of atomisation, especially as regards the viscosity of the liquid and the velocity of the air stream.

Chaussé (1914 a) studied the conditions required for atomisation of saliva and sputum into droplets small enough to form by desiccation "respirable airborne particles" (i.e. droplet-nuclei). Secretion from consumptives was subjected to artificial air-stream atomisation (by "superficial ventilation" or "deep ventilation") at various air speeds, and the exhaust atomisation air was tested for infectivity to guinea pigs which were exposed to breathe it. In the case both of saliva and sputum, air speeds less than 35 meters per second failed to atomise effectively and air speeds of 35 to 242 meters per second gave increasingly effective atomisation.

Chaussé and Magne in early studies (see Chaussé, 1914 b) estimated that during a violent cough the air speed through the trachea is from 5 to 17 meters per /

per second, and through the dilated glottis from 7 to 25 meters per second. Later, Chaussé and Magne (1916) gave higher values; the velocity of the air passing through the glottis in coughing was found often to be as high as 40 to 48 meters per second, and sometimes even 100 meters per second. Strauss (1922), using a special air-volume meter, found air speeds up to 16 meters per second in loud speaking: he recorded air-speeds of ½ to 2 m.p.s. for vowels, 2 to 3 m.p.s. for the consonants "B", "W", "G" and "D", 9 m.p.s. for "Z", 10 m.p.s. for "S", 12 m.p.s. for "K", 13 m.p.s. for "T", 15 m.p.s. for "F" and 16 m.p.s. for "P". Jennison and Edgerton (1940) and Jennison (1941, 1942) have shown by high-speed photography that droplets expelled in sneezing may have an initial velocity of up to 152 feet per second (i.e. 46 m.p.s.); the original air-stream velocities must have been greater than these droplet velocities. It appears then that in coughing and sneezing the expiratory air-stream velocities may be high enough for the production of droplets with a size-distribution similar to that found in the investigations in which atomisation was performed artificially, that is, including many droplets small enough to remain airborne as droplet-nuclei (c.f. Chaussé, 1914 a), and having a mean diameter as low as 12 microns (c.f. Sauter, 1928). However, on the grounds that the respiratory secretions are more viscous than water, Jennison (1942) has concluded that /

that respiratory droplets initially as small as about 10 microns are unlikely to be formed.

Several investigators have attempted direct measurement of the respiratory droplets by catching them on a glass slide held at a short distance in front of the mouth and measuring micrometrically the circular deposit-marks left after their evaporation (Chaussé, 1914 b; von Angerer, 1920; Hippke, 1921; Strauss, 1922, 1926); Jennison (1942) has pointed out that observations by this method do not give a true picture of the overall size-distribution of the droplets since the smallest droplets mainly escape being caught on the slide. Chaussé (1914 b), catching the droplet-spray from a consumptive on a heated (80 deg.C.) glass plate held at 15 cm. in front of the mouth, found that the droplet deposit-marks ranged from 30 to 3000 microns in diameter, mostly between 80 and 150 microns, both from coughing and from spitting violently. Hippke (1921), examining 16 patients with measles, influenza, whooping cough, diphtheria or suspected tuberculosis, caught the spray of natural coughing on slides held at 25 to 50 cm. in front of the mouth; he found that the droplet deposit-marks ranged in diameter from 30 to 3000 microns, their average diameter varying from 80 to 800 microns. Strauss (1922) caught the spray expelled in speaking by diphtheria patients and carriers on slides held at 10 to 35 cm. in front of the mouth, and found that the droplet deposit - /

deposit-mark diameters ranged from 15 to over 2000 microns, being mostly between 100 and 400 microns. Strauss (1926) caught the spray expelled in coughing on a slide held at 20 cm. in front of the mouth; he found that the deposit-marks of 258 droplets from a patient with bronchial catarrh ranged in diameter from 25 to 1700 microns, and those of 404 droplets from a consumptive from 25 to 1200 microns, in both cases the majority being between 100 and 500 microns. Strauss pointed out that the diameter of a droplet in its original spherical form is not the same as its diameter after impingement and flattening on a slide; he measured saliva drops hanging from a capillary tube, and the same drops after they had fallen on a glass slide, and so found that the diameter of the fallen drops was over 3 times larger than the diameter of the hanging drops. Allowing for the greater viscosity of sputum, he judged that the true diameter of sputum droplets is about half the diameter of the deposit-marks which they leave on a slide. Thus, he concluded that cough droplets range in original diameter mainly between 50 to 250 microns.

Valuable observations of secretion spray have been made by high-speed photography with dark-field illumination. Weyrauch and Rzymkowski (1938) obtained photographs showing the tracks of the moving droplets discharged in sneezing and speaking. Using /

Using the method of high-speed photography with stroboscopic (flash) illumination which was developed by Edgerton, Germeshausen and Grier (1937), Jennison and Edgerton (1940) were able to "stop" the motion of the droplets and, by resolving the droplets distinctly, measure their number, size, velocity and projection range. Further studies by this latter method have been reported by Jennison (1941, 1942), Turner, Jennison and Edgerton (1941), Jennison and Turner (1941) and Bourdillon and Lidwell (1941). Jennison (1942) summarised his findings. The droplets seen to be expelled by a sneeze often numbered about 20,000; a cough usually produced only a few dozen or a few hundred resolvable droplets; speaking a consonant or word produced a few dozen to a few hundred droplets, "P", "T", "F" and "S" producing the greatest numbers. In the late stages of a sneeze, the "final minimum diameters" of the droplets (presumably then nuclei) were found to range from 10 to 420 microns, with from 40 to 80% under 100 microns and from 20 to 40% under 50 microns. These observations of droplet number and size are defective in that particles smaller than 5 to 10 microns in diameter could not be resolved by the photographic method used. Many of the droplet-nuclei must have been smaller than 5 to 10 microns, since, in comparison with photographs of the early stages of a sneeze, "still" photographs taken in a late stage showed that most of the droplets had disappeared, /

disappeared, apparently because of evaporation to such small sizes that they were unresolvable; in a high-speed motion picture of a sneeze, most of the droplets had disappeared by evaporation within 0.26 seconds after the start of atomisation, and before travelling more than 3 to 12 inches from the mouth (the duration of droplet emission in this sneeze was 0.11 seconds). Neither in sneezing nor in coughing were many droplets seen to be projected further than 2 to 3 feet; in speaking, the majority of the droplets were not projected further than about 1 foot.

Jennison (1940) pointed out that in sneezing some air passes out through the nose but most escapes through the mouth, rushing at maximum speed through the approximated teeth. His photographs showed that most sneeze-droplets issued from the mouth, presumably from the front parts, and that few, if any, droplets issued from the nose; in the relatively few cases when nasal exudate was expelled, it issued as large masses and not as small droplets. In similar photographic studies, Bourdillon and Lidwell (1941) confirmed that the majority of sneeze-droplets usually originate from the mouth; in some cases, however, they found a purely nasal discharge, albeit slight, and in others a mixed oral and nasal discharge

Apart from such observations by the photographic method, information about the site of origin of secretion droplets has been obtained by experiments in /

in which a single part of the respiratory tract is artificially infected with a culture of an indicator bacterium, such as B.prodigiosus or E.coli, and the subsequently-produced droplet-spray is examined for the presence of this bacterium; the success of such experiments depends upon the tendency for the bacteria to remain localised and not to be disseminated to other parts of the respiratory tract. Teague (1913) made experiments with three subjects, on different occasions inoculating B.prodigiosus on to (1) the larynx, (2) the throat, i.e. uvula, tonsils and faucial pillars, or (3) the mouth, i.e. tongue, palate, teeth and lips; the droplet-spray from 14 coughs or 322 spoken words was caught on an agar plate held at 2 to 3 inches in front of the mouth. Teague found that in speaking most droplets originated from the mouth and few, if any, from the throat or larynx, and that in coughing many droplets originated from the larynx and from the throat, as well as from the mouth. Strauss (1922) inoculated the tonsils with B.prodigiosus; at 1½ hours later, swabs from the tonsils recovered many of these bacilli, but swabs from the mouth none; speaking during the following 5 to 15 minutes expelled many droplets, but not any which contained B.prodigiosus, except a single such droplet in the case of only 1 out of 3 persons; Strauss concluded that speech droplets originate only from the foremost parts of the mouth and that bacteria resident in the throat are /

are not likely to be expelled in speaking. Bloomfield and Felty (1924) examined two subjects, on different occasions inoculating E.coli on to (1) the throat, i.e. tonsils, posterior tongue and soft palate, or (2) the anterior mouth, i.e. lips, gums and tip of tongue; the droplet-spray from coughing or sneezing was caught on a bile-agar plate exposed at 1 foot in front of the mouth. In neither subject did the numerous droplets expelled in coughing and sneezing include any which originated from the throat; on the other hand, many droplets originating from the anterior mouth were expelled both in coughing and in sneezing by each of the subjects. Appleton and Dietz (1942) inoculated on different occasions the nose and mouth with a culture of B.prodigiosus; they found that this indicator bacillus was expelled by a snuff-induced sneeze as freely from the mouth as from the nose.

Expulsion in Droplet-spray of Specific Pathogenic Micro-organisms.

While it has been clearly established that speaking, coughing and sneezing expel large numbers of droplets, this does not afford proof that these activities expel many droplets which are infected with pathogenic microorganisms. Indeed the common finding that most or all of the expelled droplets originate from the front of the mouth, a region usually free from pathogenic microorganisms, suggests that /

that only a small minority of the droplets will be so infected. Direct observations have confirmed the paucity of droplet-spray infection.

Many studies have been made of the expulsion in droplet-spray of haemolytic streptococci. Bloomfield and Felty (1924), examining four patients with acute streptococcal tonsillitis, exposed blood-agar plates at 3 to 12 inches in front of the mouth during speaking, coughing and sneezing for 15 to 60 seconds; droplets containing haemolytic streptococci were expelled by only 1 of the 4 patients, a few during coughing and a few during sneezing. In observations carried out in a similar manner during coughing by throat carriers, droplets containing haemolytic streptococci were found to be expelled in only 1 out of 9 tests on five carriers (Colebrook, 1933), and by only 4 out of 14 carriers (Paine, 1935). Hare (1940 a) examined 12 throat carriers of haemolytic streptococci belonging to group A, exposing five blood-agar plates in a quarter-circle in front of and below the mouth, at a distance of 1 foot from it; a few droplets containing group-A haemolytic streptococci, comprising 0.3 to 3.5% of the droplets collected in the positive tests, were found to be expelled in 8 out of 37 tests of coughing six times (in all, 11 infected droplets), and in 15 out of 47 tests of speaking for one minute (in all, 27 infected droplets). None of the infected /

infected droplets were found on the plate exposed facing the mouth from a point at 1 foot horizontally in front of it; this failure of the infected droplets to be projected horizontally for so short a distance as 1 foot, suggested that these were relatively large and quickly falling under the influence of gravity. Certainly, the infected droplets appeared to be too large to remain airborne as droplet-nuclei; nuclei containing haemolytic streptococci were not recovered by the Wells air-centrifuge from the air at 1 foot in front of the mouth in seven tests of speaking for five minutes by five carriers. Hodes, Schwentker, Chenoweth and Peck (1945) on several occasions recovered haemolytic streptococci on cough plates held at 2 feet from the mouth of a scarlet-fever patient. Hamburger and Green (1946) used two methods to collect the spray expelled by carriers of the haemolytic streptococcus in coughing three times or in sneezing once: firstly, by exposing a blood-agar plate at 2 to 3 inches in front of the mouth and counting the colonies developing on this, and secondly, by holding against the face a 5-inch square muslin handkerchief and making plate-counts of broth washings from this. The average cough-plate recovery of haemolytic-streptococcus-containing droplets was 10 (0-50) for 21 nasal carriers and 2 (0-10) for 14 throat carriers, about half the plates not showing any colonies of the streptococcus./

streptococcus. The average handkerchief recovery of haemolytic streptococci from coughing was 3690 (0-47,000) for 33 nasal carriers and 1600 (0-43,000) for 32 throat carriers, most of the handkerchiefs not collecting any of the streptococci. The average sneeze-plate recovery of haemolytic-streptococcus-containing droplets was 39 (0-uncountable) for 24 nasal carriers and 8 (0-30) for 4 throat carriers. The average handkerchief recovery of haemolytic streptococci from sneezing was 2,999,000 (50-50,000,000) from the nose and 614,000 (0-4,300,000) from the mouth in the case of 18 nasal carriers, and 388 (0-3100) from the nose and 11,460 (0-106,000) from the mouth in the case of 10 throat carriers. Hamburger and Green believed that the very large numbers of haemolytic streptococci which were sneezed into handkerchiefs by nasal carriers, were contained mainly in large masses of discharged secretion rather than in droplets.

The expulsion of diphtheria bacilli in droplet-spray has been observed. Teague (1913) made 51 tests with 49 diphtheria patients who yielded diphtheria bacilli in cultures from the tonsils either at the test (40 out of 51) or previously, and many (18 of 27) of whom yielded the bacilli in cultures of the saliva. A 4-inch diameter Loeffler serum plate was held at 3 inches in front of the mouth during the speaking of about 50 words or coughing several times or crying; diphtheria-bacillus colonies /

colonies were identified by microscopical examination of methylene-blue-stained smears. Diphtheria bacilli were expelled in 28 of the 51 tests, by 65% of the patients with positive throat-cultures at the test, and on to 48 of the 180 plates. The 48 positive plates bore a total of 180 colonies of the diphtheria bacillus (3.75 per plate), 30 plates bearing 1 colony, 15 plates between 1 and 10 colonies, and 3 plates between 10 and 50 colonies. Of these colonies, 144 were from 719 coughs, and 14 from the speaking of about 2000 words. Strauss (1922) examined 2 patients and 2 carriers yielding many diphtheria bacilli in cultures from the tonsils. Microscope slides and Loeffler serum plates were held before the mouth during speaking for 3 to 10 minutes. Diphtheria bacilli were not collected in any case on the slides or plates, although these did receive a number of droplets.

Eagleton (1919) concluded from observations on carriers that meningococci are sprayed up to 13 feet by coughing and up to 5 feet during sleep. However, he neglected the possibility that his positive results were due, not to droplet-spray, but to dust-borne infection of the culture plates, as from the carrier's clothing, and indeed the circumstances under which the positive results were obtained suggest that this probably was the case. In one experiment a chronic carrier lay on a $1\frac{3}{4}$-feet high trestle in a disinfected room during 5 minutes while coughing periodically; /

periodically; out of 18 plates exposed on the floor opposite his face, 1 plate at a distance of 13 feet collected the meningococcus. In three other experiments, the meningococcus was recovered on 3 out of 18, 3 out of 18, and 6 out of 22 plates exposed between 1 and 12 feet before a sleeping carrier during 8½ hours overnight.

H.pertussis is so frequently discharged in the cough-spray of whooping-cough patients that "cough-plate" observations of the bacillus are made as a routine diagnostic test. Bradford and Slavin (1940), examining 25 consecutive cases of whooping cough, in 8 cases collected H.pertussis on a plate held 6 inches from the mouth during coughing, while recovering the bacillus in throat swabs from 4 cases and in nasopharyngeal swabs from 14 cases. Brooks, Bradford and Berry (1942), making 183 examinations on 165 whooping-cough patients, obtained H.pertussis on a cough-plate in 34% and on a nasopharyngeal swab in 57%.

In pneumonic plague, P.pestis may be spread by droplet-spray infection. Strong (1911) found that 15 of 39 plates held in front of coughing patients were infected with virulent bacilli. Wu Lien Teh, Chun Wing Han and Pollitzer (1922) allowed pneumonic plague patients to cough at agar plates held directly and perpendicularly in front of their mouths at distances of ½ to 6 feet; P.pestis was found to be sprayed out by 2 of 7 patients, being collected on plates /

plates exposed at ½ to 2 feet.

Schäffer (1898) was able to recover M.leprae from a leprosy patient by holding cover-slips at a short distance in front of the face while the patient was speaking and coughing.

Strauss (1922) found spirochaetes and fusiform bacilli in droplets caught on slides exposed to speaking by patients with ulcerative stomatitis.

Ludlam and Henderson (1942), investigating the spread of thrush among babies in a maternity hospital, exposed a blood-agar plate at 6 to 8 inches in front of the mouth of an infected baby while the latter screamed 50 times; 12 babies were examined and these gave on average less than one colony of Monilia albicans.

The expulsion of viruses in droplet-spray has not been studied directly; in a few instances there is indirect evidence suggesting that droplet-spray may be an agent of spread. For instance, Johnson and Goodpasture (1934) isolated the mumps virus from the saliva of patients up to 48 hours after the onset of parotitis, while Stallybrass (1931) noted that mumps occurring at the same time as upper respiratory infections tends to spread more rapidly and widely than ordinarily, because of the increased droplet-spray emission due to concomitant sneezing and coughing.

In conclusion, it must be emphasised that although it has been demonstrated on many occasions that /

that pathogenic bacteria are present in a small proportion of the numerous droplets sprayed out by infected persons, there is a conspicuous insufficiency of evidence that infected persons may produce droplet-nuclei which contain pathogenic bacteria. In fact, there is not any good reason for believing that droplet-spray may give rise to air infection.

High-Speed Photography of Sneezing.

(Photograph by Jennison and Edgerton, 1940) Late stage of a sneeze. Instantaneous photograph (exposure 1/15,000 second). Note tracks of moving particles beneath nose, and the string of saliva issuing from the mouth.

(7) Capacity of Respiratory-Tract Pathogenic Organisms to Survive Outside the Body.

The capacity of pathogenic microorganisms to remain alive outside the body of their host, to survive the various environmental conditions of temperature, dryness and light, determines in what ways they may be spread from person to person. In general, dryness and daylight are resisted well by spore-forming bacteria, which survive for many years, moderately well by the other Gram-positive bacteria and by some viruses which may survive for several weeks or months, and poorly by the Gram-negative bacteria, which usually survive only for a few hours.

Germano (1897 a,b,c) made the first systematic study of the capacity to survive of different bacteria; from tests in which the bacteria were dried in dust or earth, he concluded that cholera, plague, typhoid fever, influenza and gonorrhoea could not be dustborne, but that infections with streptococci, pneumococci, meningococci, diphtheria bacilli, tubercle bacilli, anthrax bacilli and tetanus bacilli, might be dustborne.

Kirstein (1902) tested the viability at room temperature of bacteria in the dried residues of small droplets (droplet-nuclei) collected from a spray into Petri dishes; the average time till death was 1 day for B.prodigiosus, 1 day for the typhoid bacillus, 1 to 2 days for the diphtheria bacillus, and 8 to 10 days for Staph.aureus.

Teague /

Teague (1913) observed the time of survival at 37 deg.C. of bacteria dried in films of saline suspensions on glass slides; the time till death was 2 minutes for V.cholerae, 28 hours for B.prodigiosus, 6 days for the typhoid bacillus, 5 days for the diphtheria bacillus and more than 7 days for a staphylococcus.

Wells and Stone (1934) observed the duration of survival in the dark, at a temperature of 70 to 80 deg.F., and at a relative humidity of less than 70%, of bacteria in airborne droplet-nuclei from sprayed broth culture; H.influenzae survived only ¾ hour, E.coli, the Hiss-Y dysentery bacillus, B.prodigiosus and Ps.pyocyanea for 4 to 8 hours, and Strept.pyogenes, Dip.pneumoniae, Staph.aureus and C.diphtheriae for 2 days.

Dunklin and Puck (1948) investigated the factors which determine the death-rate of bacteria in airborne droplet-nuclei. Working with the type-I pneumococcus (at 22 deg.C.), they found that when a suspension in broth, saliva or 0.5% saline was sprayed, a very high death-rate was observed at atmospheric relative-humidities in the vicinity of 50% (e.g. only 30% surviving for 5 minutes), while at higher or lower humidities the cocci survived for long periods (e.g. 30% surviving for 2 hours at 20% R.H., and for 1 hour at 80% R.H.). When a saline-free fluid was used, the sharp peak in death-rate at intermediate humidities was not present.

With /

With group-C haemolytic-streptococcus and with Staph.albus, the death-rates, although smaller, varied in the same way with the relative humidity of the atmosphere.

Survival of Haemolytic Streptococci.

Germano (1897 c) found that the streptococcus withstood drying for a month.

White (1936) on two occasions observed the presence of living haemolytic streptococci in the dust of a single-bed ward at, respectively, 2 and 5 days after departure of the infected patient. She quoted two tests made by Colebrook: one in which haemolytic streptococci sprayed from broth culture into dust and kept thus in a Petri dish exposed to daylight, remained alive for over 10 weeks, and another in which the streptococci were found to have retained their virulence for mice after 25 days in dust.

Van den Ende, Lush and Edward (1940) recovered living group-C haemolytic-streptococci from blankets at 2 weeks after these had been impregnated with the nasal secretions of an infected ferret, and living group-B haemolytic-streptococci from blankets at 4 weeks after spraying with a serum-broth culture; after the first 10 days of drying, the group-B cocci retained their full virulence for mice.

Thomas and van den Ende (1941) found that a sample of hospital floor-dust contained over 20,000,000 living /

living haemolytic-streptococci per gram when collected, and still about 13,000,000 living streptococci per gram after standing 2 weeks on the bench.

De Waal (1941) kept the handkerchiefs of 5 scarlet-fever patients in sealed bottles in a ward out of the sun; he found Strept.pyogenes (type 1 or 2) surviving for 3, 4, 6, 7 and 7 weeks respectively.

Buchbinder (1942) has reviewed the intensive investigations made by himself and his colleagues regarding the effect of daylight, sunlight and artificial light on the survival of streptococci. The bactericidal effect of daylight originating from a grey or blue sky, even when passing through glass, was found to be so great as to warrant it being regarded as of major hygienic importance; sunlight exerted an especially rapid killing effect; "fluorescent" artificial lighting equalled daylight in its killing power per foot-candle, but ordinarily the intensity of artificial lighting is too low for any important degree of disinfection to be obtained thereby. Broth cultures sprayed into the air were allowed to settle on sterile filter paper in open Petri dishes, which then were covered with their glass tops; after a period of exposure to daylight coming through a closed window, the dishes were filled with agar, incubated and their crop of colonies counted. The average time for 50% survival in the dark was 13 days for a group-A streptococcus and 5½ days for a group-B streptococcus /

streptococcus (Buchbinder and Phelps, 1941). The 50%-survival time in daylight (28 to 97 foot-candles) was 1-13 hours for the group-A streptococcus and ½-2 hours for the group-B streptococcus; the lower apparent death-rate of the group-A coccus was considered merely a function of its greater chain-length (Buchbinder, Solowey and Phelps, 1941). The group-B streptococcus surviving exposure in a dark room for 10 days, retained in full its virulence towards mice (Buchbinder, Solowey and Solotorovsky, 1941). Among 15 different strains of group-A streptococcus, the 50%-survival time in darkness ranged from 1.4 to 12.0 days, being on average 4.2 days, and in daylight ranged from 0.9 to 11.2 hours, being on average 4.2 hours (Solowey, Solotorovsky and Buchbinder, 1942).

Garrod (1944), in hospital wards, found group-A haemolytic-streptococci frequently to be present in the dust of floors (in 56% of 109 samples) and of skirtings (in 41% of 22 samples), but never to be present in the dust of windows and window-screens, places receiving an especial abundance of daylight (in 0% of 42 samples). Haemolytic streptococci in pus diluted 1 in 5, spread on a slide and allowed to dry, were found to survive 3 months in a dark cupboard, 2 to 3 weeks just inside a closed north window, and 1 to 2 weeks just inside a closed south-window. A sample of sifted blanket dust containing initially 204,000 haemolytic streptococci per gram, still /

still contained some of these cocci surviving after 6½ months in a dark cupboard (300 per gram) and after 2⅔ months inside a north window exposed to daylight.

Survival of Pneumococci.

Buerger (1905 b) recovered living pneumococci from a handkerchief at 7 days after it had been in use.

Wood (1905) found that pneumococci remained alive and virulent in dried sputum for an average of 35 days in the dark and 30 days in diffuse daylight. However, because she found that dried powdered sputum failed to yield viable cocci after 1 to 4 hours in the dark or 1 hour in sunlight, she discounted the likelihood of dustborne or airborne infection.

Stillman (1938) found that pneumococci of types I, II, III and VIII in dried sputum exposed at room temperature to diffuse daylight passing through glass, survived for an average of 4, 6, 8 and 4 weeks respectively; the longest survival times of the four types were 16, 17, 20 and 13 weeks.

Solowey, Solotorovsky and Buchbinder (1942), using the same technique as in the case of the streptococci, found that the average 50%-survival times of type-I, type-II and type-III pneumococci in broth-culture droplet-nuclei were, respectively, 10.8, 12.3 and 13.2 hours in the dark, and 29, 55 and/

and 42 minutes in daylight.

Survival of Staph.aureus.

Kirstein (1902) observed survival of Staph.aureus for 8 to 10 days in droplet-residues.

Wells and Stone (1934) found that Staph.aureus in broth-culture droplet-nuclei had about the same survival time as Strept.pyogenes, namely 2 days.

Survival of Meningococci.

Germano (1897 c) found that the meningococcus survived drying for 80 to 90 days; however, it has been claimed that Germano did not work with the true meningococcus.

Albrecht and Ghon (1901) found that meningococci died within 24 hours when dried on glass cover-slips and kept in the dark.

Bettencourt and Franca (1904) found that the meningococcus, when dried on glass or cotton cloth, and kept at room temperature, died within 3 to 6 hours, whether in the dark or exposed to daylight.

Von Lingelsheim (1905) found that meningococci dried on cloth did not survive longer than 12 hours at room temperature, whether in darkness or daylight.

Elser and Huntoon (1909) found that the majority of meningococcus strains died in 24 hours, but that some could survive as long as 72 hours after drying on glass.

Miller and Schad (1944 a) reinvestigated the viability of the meningococcus, using the better means /

means of cultivation then available. Living and virulent meningococci from surface culture were dried on glass beads, pieces of wood and pieces of cotton cloth. These were stored in Petri dishes with the lids ajar. The process of drying was hastened by placing the dishes in a desiccator at room temperature and atmospheric pressure during an hour or two. When the beads were dry, not sticky, the dishes were removed from the desiccator and were kept in the dark at room temperature (18 to 24 deg.C). By culture, living meningococci were recovered from glass beads up to 10 days, from wood up to 8 days, and from cloth up to 7 days. The surviving meningococci showed undiminished virulence towards mice. Miller and Schad (1944 b) found that the dried meningococci were killed by direct sunlight within a very few hours, even when protected against overheating, and were killed by diffuse winter daylight passing through glass (window and dish lid) within 30 hours on glass beads and within 5 days on wood.

Survival of Diphtheria Bacilli.

Reyes (1895) found that diphtheria bacilli remained alive and virulent in sand or on cloth for 14 days.

Germano (1897 b) found that diphtheria bacilli retained their virulence in dry earth or dust for 20 to 40 days.

Buckley /

Buckley (1906-07) recovered living diphtheria bacilli after 6 days when dried in the air on paper, after 8 days on wood, after 24 days on cotton or glass, and after 37 days on plaster.

Hill (1902) found that diphtheria bacilli survived exposure to ordinary room conditions for 20 days on 9% of glass rods which had been smeared with culture.

Crosbie and Wright (1941) collected ten samples of infected dust from the floors of wards in fever hospitals. These were stored in test tubes which were shielded from direct light but not darkened. Most of the samples still contained living and virulent diphtheria bacilli after 1, 2 or 3 months. Survival under natural conditions on the ward floor was observed over 1 month while the ward was occupied exclusively by non-carriers.

Survival of the Anthrax Bacillus.

The causation of pulmonary anthrax by inhalation of dustborne spores is well known. Graham-Smith (1930) found that if spores of B.anthracis were kept at room temperature and exposed to diffuse daylight, about 50% died within a few months, but a proportion remained alive for many years. After 22 years of storage, 14 pieces of spore-impregnated cloth were examined by culture; 11 pieces were found to be sterile, while the remaining 3 pieces gave, respectively, 1, 1 and 3 colonies of B.anthracis.

Survival /

Survival of the Plague Bacillus.

Although Gram-negative, P.pestis is sufficiently resistant to light and drying for pneumonic plague commonly to be dustborne.

Rosenau (1901) found that P.pestis did not survive in dry bone dust for more than 6 days.

Tidswell (1902) found that when dried in dust under natural conditions, P.pestis survived only for 3 to 4 days, but that it lived for 3 weeks when dried slowly on muslin.

Wu Lien Teh, Chun Wing Han and Pollitzer (1922) observed the survival of P.pestis in sputum from pneumonic plague patients. In sputum stored in the dark at -3 deg.C., the bacilli survived for 99 days, but not for 208 days. In sputum exposed to diffuse daylight through glass and kept at 0 to 10 deg.C., the bacilli survived for up to 48 hours. In sputum exposed to sunlight through glass, the bacilli survived for 2 hours when at temperatures above 12 deg. C., and for up to 8 hours when at -3 deg.C.. In sputum dried on wood at 10 deg.C., the bacilli survived for 3 days.

Survival of H.influenzae.

Pfeiffer (1895) found that H.influenzae survived for 36 to 40 hours when dried in sputum, but only for 8 to 20 hours when dried on a cover-glass at room temperature.

Survival /

Survival of Rickettsia burneti.

Derrick (1943) has suggested that inhalation of tick faeces containing R.burneti is the most likely mode of acquisition of Q fever. In two experiments, infected tick faeces retained their infectivity for 65 and 87 days.

Survival of the Small-Pox Virus.

It is thought that small-pox is acquired by inhalation. The virus is sufficiently resistant to drying for dustborne infection to be common.

Brinckerhoff (1904) found that the virulence of small-pox crusts persisted for periods of 22 to 88 days.

Downie and Dumbell (1947) by egg-culture showed that small-pox virus survived in vesicle fluid dried on glass slides for 35 days when exposed to daylight and for 84 days when kept in the dark at room temperature. The virus was found to survive for several months, in one case for over a year, in crusts which were kept at room temperature and either in light or in darkness.

Buchbinder and Solotorovsky (1941) atomised a suspension of vaccinia virus; living virus was recovered from the air for 8 hours.

Survival of the Influenza Virus.

Edward (1941) found that when a suspension of influenza virus was dried on cloth, dust or glass, between /

between 1 and 10% of the virus survived the process of drying under ordinary room conditions, and some virus thereafter remained alive for more than 2 weeks in the dark, and for 2 days when exposed to daylight passing through glass.

(8) Occurrence of Pathogenic Microorganisms in Personal and Environmental Reservoirs and Vehicles of Infection.

The extent to which an infected person charges the various objects and surfaces of his environment with pathogenic microorganisms determines the main routes by which infection may be transmitted to other persons. Contamination of lips, hands, fomites and eating utensils determines the likelihood of spread by contact to the mouth, and contamination of clothing, bedding, handkerchiefs, floor dust and furniture dust determines the likelihood of dust-borne transmission through air, leading to infection by inhalation.

Pathogenic Bacteria on the Lips.

Hare (1941) examined on several occasions each of 7 nasopharyngeal carriers of Strept.pyogenes; he cultured the streptococcus on some occasions from the upper lip of 4 of the 7 carriers and from the lower lip of 3 of the 7 carriers.

Pathogenic Bacteria on the Hands.

Colebrook, Maxted and Johns (1935) cultured beta-haemolytic streptococci from the hands of 17 out of 181 normal persons; of these strains, 7 belonged to group A, 1 to group C, 1 to group F, 1 to group G and 3 to group H; thus, 3.8% of healthy persons carried Strept.pyogenes (group A) on their/

their hands, probably as transient contaminants derived from their true source in the nasopharynx.

Hare (1941) cultured Strept.pyogenes (group A) from the hands of only 1 out of 248 healthy persons; the contaminated person was not carrying the coccus in his throat, but apparently had received it on his hands from a room mate who was known to have had a throat infection with the same serological type of coccus. Hare also examined 7 nasopharyngeal carriers of Strept.pyogenes (group A) and cultured this coccus from the hands of 2 out of the 7 carriers.

Hamburger and Green (1946), making cultures of 1-minute broth washings of the hands, recovered living haemolytic streptococci (in most cases, of group A) from the great majority of 106 nasal carriers and from 60% of 74 throat carriers with negative nose cultures. The number of haemolytic streptococci recovered from the pair of hands ranged from 0 to 21,000,000 and averaged 790,000 among the nasal carriers, and ranged from 0 to 250,000 and averaged 4700 among the throat carriers. An average of 448,000 haemolytic streptococci were at once put on to the hands by a single act of blowing the nose into a sterile muslin handkerchief, in the case of 29 nasal carriers tested.

Allison and Hobbs (1947), investigating hospital outbreaks of Pemphigus neonatorum, cultured Staph.pyogenes from the hands of 28 out of 103 members/

members of the nursery staffs; 15 of the 28 strains belonged to the same serological type as that causing the infections among the infants. The staphylococci on the hands probably were derived from the nose, since all the hand carriers were found also to be nose carriers.

Ludlam and Henderson (1942), investigating the spread of neonatal thrush in a maternity hospital, found the causative organism, Monilia albicans, on the hands of 9 out of 18 infants with oral infection, on the hands of 0 out of 11 infants without oral infection and on the fingers of 3 out of 42 nurses attending the infected infants.

Pathogenic Bacteria on Clothing.

Hare (1941) cultured haemolytic streptococci of groups B, C and G from the trouser legs of 5 out of 121 healthy persons, but Strept.pyogenes (group A) from none. Examining 7 nasopharyngeal carriers of Strept.pyogenes, Hare cultured this streptococcus on some occasions from the tie of 3 out of the 7, from the shirt front of 2 out of 4, from the coat lapel of 2 out of 7, from the shirt back of 0 out of 3, from the coat back of 1 out of 7, from the handkerchief pocket of 3 out of 7, and from the trouser leg of 1 out of 7. Hare concluded that Strept.pyogenes rarely is present on the clothing of normal persons, but may be present extensively on the clothing of nasopharyngeal carriers.

Hare/

Hare and Mackenzie (1946) made experiments which showed that the clothing readily becomes contaminated with bacteria from the upper respiratory tract. A culture of B.prodigiosus was instilled into the nose or mouth of a normal person, and after 1 hour during which the subject took sneezing powder and carried on his ordinary occupation, plate-counts were made on broth washings of 1-inch square lint patches from various parts of his clothing; B.prodigiosus was recovered from the lapels, sleeves, abdominal region and back of the clothing in numbers ranging from 10 to 1000 per square inch, both following infection of the mouth and following infection of the nose.

Colebrook and Ross (1947) recovered viable haemolytic streptococci from the clothing of all of 4 throat carriers, and also from the clothing of 3 out of 8 non-carriers working in contact with infected patients. The haemolytic streptococcus was cultured from the waistcoat of a surgeon having a small scabbed sore on his elbow which harboured this bacterium.

Bourdillon and Colebrook (1946) found that pyjama jackets and night gowns became heavily contaminated with bacteria, including Staph.aureus in some cases, within so short a period as 3 hours of a patient putting on the garment for the first time.

Wu Lien Teh, Chun Wing Han and Pollitzer (1922) cultured P.pestis from 14 out of 30 pieces of cloth cut/

cut from the coat fronts of newly dead pneumonic-plague patients.

Pathogenic Bacteria on Handkerchiefs.

De Waal (1941) cultured Strept.pyogenes from the handkerchiefs of 5 patients with scarlet fever.

Hare (1941) cultured Strept.pyogenes (group A) from the handkerchiefs of 3 out of 6 nasopharyngeal carriers.

Hamburger and Green (1946) found that a nasal carrier in blowing his nose 3 times put into his handkerchief between 10,000 and 10,000,000 haemolytic streptococci in 73% of cases, and over 1,000,000,000 in rare instances.

Buerger (1905 b) cultured pneumococci from a handkerchief.

Jaeger (1894) is quoted by Germano (1897 d) as having found the meningococcus in a handkerchief at 6 weeks after its being used by a patient with cerebro-spinal meningitis.

Trevelyan (1900) recovered diphtheria bacilli from a handkerchief at 11 weeks after it had been used by a patient.

Pathogenic Bacteria on Bedding.

Hare (1941) cultured Strept.pyogenes (group A) from the bed-sheets and pillow of a nasal carrier, who also had suffered a streptococcal paronychia, but not from the bedding of two other nasal carriers and/

and two throat carriers.

Thomas and van den Ende (1941) used a special extractor for washing a portion of a blanket with broth. They found that the blankets of tonsillitis patients in a multi-bed ward each contained between 500,000 and 1,000,000 living haemolytic streptococci. Within 48 hours after a tonsillitis patient had been transferred to clean bedding, his blankets each had collected 400,000 haemolytic streptococci, and his counterpane 1,000,000.

Colebrook, Gibson and Todd (1944), investigating the infection of burns in Glasgow Royal Infirmary, found that, to avoid shrinkage, bed-blankets were laundered by rinsing in water not hotter than 42 deg.C.; on two occasions, haemolytic streptococci of the type infecting the burns were isolated from clean, laundered blankets in the linen store.

Garrod (1944) found 204,000 living haemolytic streptococci per gram in vacuumed blanket dust filtered through gauze.

Hamburger, Puck, Hamburger and Johnson (1944) found that cultures of sheets, pillow-cases and blankets yielded hundreds or thousands of haemolytic streptococci as soon as 6 hours after their first use by certain patients with streptococcal infection of the upper respiratory tract.

Hamburger, Green and Hamburger (1945 a) found that the daily discharge of haemolytic streptococci on/

on to a 25-sq. in. "patch" on the bottom sheet of the bed was, in the case of tonsillitis and scarlet fever patients with strongly positive nose cultures, on average about 50,000, ranging between 1000 and 100,000 in over 70% of cases, and up to a maximum of 600,000, and, in the case of patients with negative or weakly positive nose cultures, on average about 700, being less than 1000 in 80% of cases, and never exceeding 6200.

Allison and Hobbs (1947), investigating the spread of Pemphigus neonatorum in a maternity hospital, cultured Staph.pyogenes from 5 out of 6 blankets from infants' cots; Staph.pyogenes was again isolated from one blanket when this was re-examined after laundering, which process was apparently inadequate to kill the cocci.

Park (1892) found the diphtheria bacillus in almost every instance in dried stains on bed-clothing soiled by patients. Kober (1899) failed to find this bacillus on the bed-linen in houses where there had been diphtheria.

Pathogenic Bacteria in Floor Dust.

Horwood (1931) examined samples of dust from family residences, shops, offices and institutions; haemolytic streptococci were cultured from 20 out of 45 samples and haemolytic staphylococci from 21 out of 45 samples.

Cruickshank/

Cruickshank (1935) found numerous haemolytic streptococci in the dust of burn wards in which there was a high incidence of streptococcal infection of the burns and also of the patients' throats.

Brown and Allison (1937) cultured Strept.pyogenes from the floor dust of scarlet-fever wards.

Hare (1941) cultured Strept.pyogenes (group A) from the carpets of the bedrooms of two nasal carriers, but not from the carpets of another nasal carrier and two throat carriers.

Thomas (1941) examined the dust swept up one morning from the floors of a hospital unit housing 22 patients of whom 19 were infected in the throat with Strept.pyogenes. The total of 29.06 gm. of dust contained 102,000,000 Strept.pyogenes, that is over 3,000,000 per gram. Of these, 16,261,000 were from a 16-bed ward, 23,323,000 from a 10-bed ward, 17,020,000 from a 1-bed cubicle, 1,173,000 from a second cubicle, 160,000 from the surgery, 1,411,000 from the main corridor, 1,041,000 from the office, 939,000 from the linen room, 329,000 from the staff room, 320,000 from the day room, 1,171,000 from the entrance corridor, and 38,745,000 from the bathroom and lavatory annexe. It may be suggested that this very high contamination of floor dust in the lavatory premises is due to the very frequent doffing and donning of clothes which occurs there.

Glass (1941) cultured Strept.pyogenes of the infecting/

infecting type from the dust of a 1-bed ward at 8 days after a puerperal fever patient had left the ward and the ward had been subjected to washing and treatment with formalin.

Walter and Hucker (1942) cultured beta-haemolytic streptococci from the floor dust of 22 out of 37 rooms investigated in 6 schools, a boys' dormitory, a theatre and a hotel. Of 17 strains tested serologically, 7 belonged to group A, 2 to group B, 1 to group C, and 7 probably to group G.

Garrod (1944) found Strept.pyogenes (group A) in 72% of 76 dust samples from a ward receiving little daylight and in only 18% of 33 dust samples from a ward receiving much daylight. In an 18-bed ward a few Strept.pyogenes were found in nearly every specimen of floor dust, and large numbers were found in specimens taken from near the beds of four patients with known Strept.pyogenes infection of wound or throat.

Edward (1944) during 6 months made a weekly examination of the floor dust of two multi-bed ward units with adjacent 1-bed cubicles; these housed patients who included a large proportion of Strept.pyogenes (group A) carriers. The ear, nose, throat and eye unit yielded on average 300,000 living haemolytic streptococci per gram (33% being group A); of 99 samples, 83 contained between 1000 and 1,000,000 per gram and 10 over 1,000,000 per gram.

The/

The children's ward yielded on average 250,000 living haemolytic streptococci per gram (80% being group A); of 223 samples, 165 contained between 1000 and 1,000,000 per gram, and 6 over 1,000,000 per gram; on one occasion, 16,000,000 per gram were found in a 1-bed cubicle.

Hamburger, Puck, Hamburger and Johnson (1944) cultured 85 dust samples (about 0.25 gm.) each collected, at ½ to 48 hours after "dry sweeping", from 28 sq. ft. of floor under and around a patient's bed in a measles or respiratory disease ward where cross-infections with haemolytic streptococci were occurring; 83 of the 85 samples yielded living haemolytic streptococci in numbers ranging from 150 to 1,755,000, with about 75% of the samples containing between 1000 and 1,000,000; the streptococci comprised about 0.1% of the total bacterial flora of the dust.

Netter (1888, 1897) recovered pneumococci from the dust of hospital wards.

Washbourn, White and Eyre (1902) by mouse inoculation recovered pneumococci from the dust of a ward in which pneumonia patients were nursed, but not from dust obtained from other parts of the hospital or from the street.

Stillman (1917) found pneumococci in 74 (i.e. 40%) of 183 dust samples from houses in which there had been cases of pneumonia (25 of the positive samples/

samples contained type-I cocci and 23 contained type-II cocci), and in 18 (i.e. 29%) of 62 dust samples from other houses (only 1 of the positive samples contained type-I cocci).

Jaeger (1899) claimed to have found meningococci on the floor of an army barracks.

Wright, Shone and Tucker (1941) cultured diphtheria bacilli, often in large numbers, from 16 out of 17 specimens of dust swept from the floors of four diphtheria wards.

Crosbie and Wright (1941), using Hoyle's medium, cultured virulent diphtheria bacilli, in small or large numbers, from 37 out of 44 samples of dust from the floors of wards in fever hospitals, and from 14 out of 32 samples of dust from the floors of wards in a general hospital; the frequency of the different types of the bacillus, gravis, intermedius and mitis, occurring in the dust, corresponded to the incidence of the different types in patients and carriers.

Allison and Hobbs (1947) examined two samples of dust collected on different days from the floor of a nursery in a maternity hospital having a high incidence of staphylococcal infections; cultures yielded large numbers of organisms, mainly saprophytic, but Staph.pyogenes was present in considerable numbers in one sample, and in small numbers in the other.

Robinson/

Robinson, McLeod and Downie (1946) isolated typical toxigenic Cl.tetani from the floor dust of two operating theatres where there had been cases of post-operative tetanus, and from the dust of 1 of 11 other operating theatres in 3 hospitals.

Downie and Dumbell (1947) by egg culture isolated living Variola virus from dust swept from the floor near a bed in which a child was convalescing from small-pox.

Pathogenic Bacteria on Fomites.

Hilditch (1908) examined 24 paper-money notes and found that the number of bacteria varied from 14,000 to 586,000 per note; bacteria were not numerous on coins, chiefly because of the germicidal action of the metal. Hilditch reported that there was not any evidence to suggest that employees in the U.S. treasurer's department contract infectious diseases especially frequently.

Brown and Allison (1937), De Waal (1941) and Wright, Cruickshank and Gunn (1944) frequently cultured Strept.pyogenes from toys, pencils, beads, bibs, books, lockers, tables and baths which had been used by scarlet-fever patients or carriers of the streptococcus.

Miles and his colleagues (1940) obtained copious growths of Strept.pyogenes and Staph.aureus from the inside of ward baths which were used for infected wound cases; the bacterial flora was richest in the line/

line of grease left at the level of the water surface.

Abel (1892) found diphtheria bacilli on toys at 86 days after infection.

Williams (1895) recovered diphtheria bacilli from pencils moistened by the lips of children with diphtheria.

Schumburg (1905) in 40 cultures from a diphtheria patient's room recovered virulent diphtheria bacilli from a drinking glass and from the handle of a mirror.

Klein (1905) did not find diphtheria bacilli on 12 telephones in London.

Hill (1906) took 532 swabbings from the room of a diphtheria patient and recovered diphtheria bacilli 4 times, in each case from an object handled by the patient.

Pathogenic Bacteria on Eating Utensils.

Cumming (1917) claimed to have isolated haemolytic streptococci from 91% of specimens from 23 sets of tableware, and diphtheria bacilli from 2% of specimens from 26 sets of tableware.

Saelhof and Heinekamp (1920), examining 63 eating and drinking utensils, cultured haemolytic streptococci of the human type from 6.4%, and Staph.aureus from 3.2%.

Lyons (1936) discussed the possibility that Vincent's infection (trench mouth) is spread by use of improperly cleansed beverage glasses in taverns and saloons/

saloons. By microscopical examination of swabbings from 200 glasses from 8 establishments, he found Borr.vincenti on 24 glasses, that is 12%. In one establishment, Borr.vincenti was present on 8 of 62 glasses examined after use by patrons and before rinsing, and on none of 30 glasses examined after they had been washed mechanically in hot water with a chlorine preparation added. In a second establishment Borr.vincenti was present on 3 of 22 glasses examined just after use, and on 4 of 20 glasses which were stacked for reissue, supposedly having been cleaned.

Horwood and Pesare (1942) emphasised the great extent to which public eating and drinking establishments are patronised; according to Calver, the number of persons in the United States making use of these establishments is equivalent to the service of one meal or drink per person per day. Horwood and Pesare examined utensils from 55 establishments, including restaurants, soda fountain dispensaries, cafés and barrooms; among 184 utensils, including plates, tumblers, spoons, forks, beer glasses and rinse waters, haemolytic streptococci were found on 1 plate and 1 spoon (i.e. on 1.1% of utensils) and haemolytic Staph.aureus on 2 plates, 5 tumblers, 1 spoon and 2 forks (i.e. on 5.4% of utensils), while the diphtheria bacillus, the tubercle bacillus and Vincent's organisms were not found on any utensil (0%).

Hutchinson/

Hutchinson (1947) visited 25 kitchens in hotels, restaurants, works and school canteens, snack bars and day nurseries, and swabbed utensils which had been used, washed and stacked for reissue. Among 164 spoons, 121 cups, 219 forks, 53 plates and 58 glasses, Staph.aureus (coagulase positive) was found on 8 spoons, 9 cups, 8 forks, 6 plates and 3 glasses (i.e. on 5.5% of utensils), Strept.pyogenes (group A) on 1 fork (i.e. on 0.16% of utensils), haemolytic streptococci of groups B, C or G on 2 spoons, 4 cups, 1 fork, 2 plates and 4 glasses (i.e. on 2.1% of utensils), coliform bacilli on 27 spoons, 30 cups, 43 forks, 23 plates and 10 glasses (i.e. 21.6% of utensils), and the Sonne-type dysentery bacillus on 1 spoon (i.e. 0.16% of utensils). The temperature of the dish-washing waters ranged from 30 to 50 deg.C., and thus were too low for killing of the contaminating bacteria.

Pathogenic Bacteria on Flies.

Graham-Smith (1910) has shown that in addition to transporting bacteria on their feet and wings, house-flies, after feeding on some infective discharge, may distribute the bacteria in their faeces and in the frequent regurgitations from their crops. In the case of flies fed on a culture suspension, diphtheria bacilli seldom remained alive on the legs and wings for more than a few hours, but survived in the alimentary canal for a day or so, and were/

were excreted alive in the faeces for up to 51 hours. In the case of flies fed on infected saliva, the diphtheria bacilli survived in the crop and intestine for up to 24 hours, and were excreted alive in the faeces for up to 6 hours.

Shooter and Waterworth (1944) in two surgical wards trapped 27 flies in culture plates and allowed them to wander over the surface of the medium until death; 9 of the flies gave growths of a haemolytic streptococcus, in 3 cases this being Strept.pyogenes (group A); Staph.aureus (coagulase positive) also was cultured from some of the flies. Two of the group-A streptococcus strains were of the same serological type as a strain which had been cultured from a nurse's throat some ten days previously.

(9) Mechanism of Transmission by Contact with the Hand.

It may be concluded from the many investigations which have just been reviewed, that pathogenic bacteria from the respiratory tract are present fairly commonly on the hands of nose carriers and throat carriers. This has led to the widely held assumption that such bacteria are readily transmitted by contact to other persons, for instance on to their hands in hand-shaking. However, experimental evidence has been obtained which indicates that only a very few bacteria are likely to be transmitted in this way; for physical reasons, only a few bacteria are transferred between two dry surfaces in contact; moreover, the rapid autodisinfecting action of the human skin limits the opportunity for transmission of living bacteria.

Ostermann (1908) spread over the palm of his left hand two drops of ascitic fluid containing 150,000 to 100,000,000 spores of a saprophytic bacillus. After drying for about 30 minutes, he pressed the thenar eminence of his right hand on the palm of his left hand, lightly as in hand-shaking. By making plate-cultures of washings from his right hand, he found that the number of spores transferred from hand to hand, ranged, in the various experiments, from 1 out of 3000 to 1 out of 200,000 of the spores originally present on the infecting left hand. In

experiments using sputum instead of ascitic fluid as a vehicle for the spores, the largest number of spores transferred from hand to hand was 1 out of 22,000; exceptionally, in warm weather, or in the case of persons suffering from moist hands, up to 1 in 15,000, and even 1 in 2700 were transferred.

Apart from direct hand to hand contact, bacteria might be transmitted more indirectly, as from the infector's hand to a door-knob, chair-back, telephone-handle or other object, and from the latter to the recipient's hand. Also, the recipient's hand might be infected by touching the infector's clothing or any object contaminated with airborne dust-particles or projectile secretion-droplets derived from the infector.

Whatever the mechanism may be, there is evidence that persons do, under natural conditions, pick up pathogenic bacteria on their hands. Hare (1941) found Strept.pyogenes on the hands of a person who was not carrying the streptococcus in his respiratory tract, and who must have received it from a room-mate known to be suffering a throat infection with this particular serological type of streptococcus. Ostermann (1908) similarly found tubercle bacilli on the hands of several healthy persons, children and a nurse, who were in contact with consumptives.

The autodisinfecting activity of the human skin against/

against certain species of pathogenic bacteria, by attacking such bacteria during their residence on the hands of the infector and the hands of the recipient, must limit to an important degree the number of living bacteria which may be transmitted by hand contact.

Colebrook (1930), after smearing the finger with a broth culture of haemolytic streptococcus and allowing 3 minutes for drying, recovered on immediate swabbing 30,000,000 living cocci, on swabbing after 1 hour only 1,722,000, and after 2 hours only 7000.

Arnold and his colleagues (1930) found that when B.prodigiosus was placed on the skin of the hands, only 10% could be recovered after 10 minutes, 1% after 20 minutes, and, in most cases, none after 30 minutes. In contrast, when Staph.aureus was applied to clean palmar skin, 18% were recovered after 10 minutes, and some were recovered after much longer periods. Dirty skin, and certain regions such as the lateral margins of the nails and the region under the nail tip, freed themselves of bacteria much more slowly than clean palmar skin.

Burtenshaw (1945) has summarised the mechanisms of skin autodisinfection; the bacteria are killed by drying, by light, by the acidity of the sweat and by the long-chain fatty-acids of the skin secretions. The pH of the sweat is usually between 4.0 and 6.0; the higher acidities within this range are likely to be/

be lethal to bacteria such as the haemolytic streptococci. Burtenshaw (1942) found that ether and alcohol extracts of human skin and hair had a powerful bactericidal action towards the haemolytic streptococcus, Strept.viridans and the diphtheria bacillus, only a weaker and less constant effect on the pneumococcus, Staph.aureus and Staph.epidermidis, and no effect on the typhoid bacillus and the colon bacillus. By fractionation of the extracts it was found that the bactericidal activity was due to long-chain fatty-acids (chains of 10 or more carbon atoms), especially to unsaturated fatty-acids such as oleic acid. The soaps, the form in which the fatty acids would occur at pH 7.0, possessed some bactericidal activity, but not so much as the free fatty acids at pH 5.0. Presence of blood inhibited the streptococcicidal action of the fatty acids.

Burtenshaw (1938) studied the relative importance of drying as compared with the other factors, in fixing and killing bacteria applied to the skin. Staph.aureus was resistant both to drying and to the other autodisinfecting mechanisms; of staphylococci applied in a distilled-water suspension to the skin of the palm, about 50% were recovered alive after drying for 5 minutes and about 35% after 1 hour. The haemolytic streptococcus was much more susceptible; of the streptococci applied, the average number recovered after 5 minutes of drying

was/

was 9% from the palm, 20% from the fingers, 6% from the forearm, 6% from glass and 7% from fine rubber; apparently the reduction during this period was due mainly to the drying, since it was as great on glass and on rubber as on the skin. The number of living streptococci recovered after 1 hour was, on average, 0.7% from the palm, 5% from the fingers, 2.7% from the forearm, 1.3% from glass and 3% from rubber; the exceptionally great reduction on the palm must have been due to some bactericidal mechanism other than drying, in fact to autodisinfection by the skin.

(10) Methods of Bacteriological Examination of Air.

The different methods which may be employed for examining the bacterial content of the air, have been reviewed by Annand and his colleagues (1941), by Bourdillon, Lidwell and Thomas (1941), and by Du Buy and Hollaender (1945).

In almost all methods the bacteria are observed and enumerated by culture and colony counting; only the living bacteria are thus revealed. A few of the early investigators, including Pasteur (1860), observed the bacteria microscopically.

There are two main differences of procedure according to which the various methods may be classified. Firstly, the method of collection of the bacteria-carrying particles from the air may be either: (1) by exposing horizontal "settling dishes" to catch the large airborne particles which settle downwards under the influence of gravity, or (2) by drawing a measured volume of air through an "air sampling device" which extracts from it both large and small particles. Secondly, the method of counting the collected bacteria may involve either: (1) using the surface of a solid agar medium for receipt of the airborne particles and counting the colonies which develop on this after incubation, or (2) using a liquid medium for receipt of the airborne particles and counting the colonies in poured-plate cultures made from portions of this liquid.

Simple/

Simple exposure of "settling dishes" is the easiest and most convenient method of observing the bacterial content of the air, but it yields only qualitative information. In order to obtain an absolute measurement of the amount of air infection, it is necessary to use an "air sampling device" which extracts the particulate content from a known volume of air.

Colony counts obtained after collection of the airborne particles on to the surface of a solid agar medium, represent the numbers of bacteria-carrying particles collected from the air, and not the total number of bacteria, since many bacteria may be present as a cluster in a single particle. Plate-counts made from liquid medium into which the air-borne particles have been collected, represent neither the number of bacteria-carrying particles collected nor the total number of bacteria, but a number intermediate to these, since the bacterial clusters on each particle break up to a partial and indeterminate extent when collected in the liquid; for this reason, because the counts given are of uncertain significance, methods involving collection into liquid, although they give higher counts, are less valuable than methods involving collection on to a solid agar medium.

"Settling Dishes".

Exposure of open "settling dishes" is so simple

a/

a procedure that it is still widely used for observation of the kinds of bacteria present in the air. The main shortcomings of the method are that it collects efficiently only the larger airborne particles, and that it gives counts which have comparative value only, being unrelated to any known volume of air.

According to Wells, Winslow and Robertson (1946) and Bourdillon and Lidwell (1948 b), "settling dishes" collect particles smaller than 10 to 20 microns in diameter inefficiently and in relatively small numbers as compared with the larger particles. Bourdillon, Lidwell and Thomas (1941) compared "settling dishes" with the highly efficient "slit sampler". The "slit sampler to settling dish ratio", that is the ratio of the volume count per cu. ft. to the area count per $3\frac{1}{2}$-inch diameter dish per minute, was 5.5 to 1 when sampling the air of a crowded room containing many large infected dust particles, and from 1000 to 1 to 5000 to 1 when sampling air containing minute infected droplet-nuclei produced by finely spraying a suspension of staphylococcus or streptococcus in distilled water. Thus, the "settling dish" was found to be more than 200 times as efficient in collecting from the air the large bacteria-carrying particles as in collecting the smallest bacteria-carrying particles. This is an important defect, because under some circumstances the/

the small particles may carry different kinds of bacteria from the large.

If "settling dishes" are exposed for equal periods of time, either at different times or in different places, the counts obtained on the different plates will indicate comparatively, but not absolutely, the different amounts of air infection occurring at the different times and places.

Derivation of an absolute measurement of air infection from "settling dish" counts, has been attempted. Petri is quoted by Masterman (1941) as saying that the bacteria present in 10 litres of air are deposited on 100 sq. cm. of a "settling dish" in the course of 5 minutes (i.e. from $2\frac{1}{2}$ cu. ft. on to a $3\frac{1}{2}$-inch diameter dish per hour). This equivalence, however, must be very approximate and can not be of much value. In any case, it can not cover the bacteria on the small particles which do not settle.

Winslow and Robinson (1910) developed a modification of the "settling dish" method whereby an absolute measurement of the amount of air infection might be obtained. The "settling dish" is covered with a cylinder enclosing 1 litre of air and allowed to stand thus for 30 minutes, a time considered sufficient for sedimentation of all the bacteria in the enclosed litre of air. The drawback to this method is that a large enough volume of air can not be sampled.

A/

A modern use of the "settling dish" is that developed by Wells and his colleagues for estimation of equivalent particle diameter from the ratio of "volume count per cu. ft." to "area count per sq. ft. per minute"; equivalent particle diameters calculated in this way, included 2.6 microns for droplet-nuclei from atomised culture suspension, 13.5 microns for sneeze-produced droplet-nuclei, and mainly from 10 to 30 microns for the bacteria-carrying particles occurring naturally in various indoor atmospheres (Wells, Phelps, Robertson and Winslow, 1940; Wells, 1943; Wells, Winslow and Robertson, 1946).

Air Filtration Samplers.

In samplers which operate by filtration of the air, known volumes of the air are drawn through a porous solid filtering material, this material is washed out in a liquid such as broth, and the bacteria acquired by the latter are enumerated by the making of plate-counts.

Pasteur (1860) used a water aspirator to draw the air through a small plug of nitrocellulose "wool"; the "wool" was dissolved in an alcohol-ether mixture and, after sedimentation, the bacteria and spores were taken for microscopical examination.

Frankland (1887) aspirated the air through a 5-inch long, $\frac{1}{4}$-inch diameter glass tube containing glass wool and granulated sugar as the filter material.

Petri/

Petri (1888) aspirated the air through a glass tube filled with sand; the sand was shaken with nutrient gelatine and plated.

Bourdillon, Lidwell and Thomas (1941) point out that when sand, cotton-wool or sintered glass is used as the filter material, very thorough washing is required to ensure removal of most of the bacteria; when a soluble salt or sugar is used, this difficulty does not arise, but inhibition of bacterial growth may be caused.

Air Bubbler Samplers.

In the bubbler type of air sampler, a known volume of the air is drawn in the form of fine bubbles through a column of liquid such as broth, saline or distilled water; plate-counts on this liquid reveal the number of bacteria collected. Unfortunately, a considerable proportion of the smaller suspended particles may escape being trapped in the liquid, especially when the bubbles are too large.

In his classical experiments on droplet-spray infection, Laschtschenko(1899) drew the air through sterile saline by means of a water pump or hand bellows, measuring it with a gas meter; an hour was required for sampling of a cubic meter of air.

Rettger (1910) described an "aeroscope" in which the air was bubbled from a perforated bulb at the end of the air-inlet tube, through sterile water in a container surrounding the bulb; the air was sampled at/

at only half a litre per minute, a rate which now would be considered entirely inadequate for air hygiene investigations.

McConnel and Thomas (1925) devised a silk bubbler "aeroscope" in which a layer of fine mesh silk over the immersed end of the air-inlet tube acts as a filter and ensures division of the air into very fine bubbles; air was sampled at the slow rate of 7 to 14 cu. ft. per hour. Bourdillon, Lidwell and Thomas (1941) compared this sampler with the highly efficient "slit sampler"; parallel sampling showed that the "silk bubbler" had a relative overall sampling efficiency of only 18% for the naturally infected air of a crowded room; tandem sampling of a fine spray from a bacterial suspension in distilled water, showed that the "silk bubbler" had a collection efficiency of 60 to 90%, but the collected bacteria could not all be grown.

Wheeler, Foley and Jones (1941) developed a "bead bubbler" sampler in which known volumes of air are drawn through a cylinder containing broth and glass beads; the beads ensure division of the air into fine bubbles, and thus intimate contact with the broth.

Moulton, Puck and Lemon (1943) designed an "atomiser bubbler" in which the air being sampled is used to atomise a portion of the collecting broth, so to contact it intimately, and the exhaust spray is collected/

collected by bubbling it through a second portion of broth.

Lemon (1943) designed a "Folin-tube bubbler" which he found to be 90% efficient in comparison with the 95% efficient Moulton "atomiser bubbler". The air is drawn at the high rate of 30 litres per minute through the six lateral holes of a Folin aeration tube into the collecting broth at the bottom of a test tube; the rapid air flow, which causes the instrument to act as an impinger rather than as a bubbler, was thought to be the reason for the high efficiency.

Bourdillon and Lidwell (1948 a) compared a "bead bubbler" and the Lemon "Folin-tube bubbler" against the highly efficient "slit sampler". Sampling potassium bromide droplet-nuclei of mean diameter estimated at 6.5 microns, the collection efficiency of the "bead bubbler" was found to be 74% on average, and that of the Lemon "Folin-tube bubbler" to be 96%. Sampling finely sprayed Strept.salivarius, the overall efficiency of the "bead bubbler" was found to be 66%, and that of the "Folin-tube bubbler" to be 104%.

Rosebury (1947) described a simple "capillary impinger" for sampling experimental atmospheres containing high concentrations of bacteria. The air is drawn at 2 to 3 litres per minute through a standard capillary tube dipping 5 mm. below the surface/

surface of the collecting liquid in the bottom of a flask. Although constructed like a bubbler, this sampler operates as an impinger.

Elliott (1941) has advised a "steam jet sampler" in which the air is filtered by drawing it through a mist of water vapour, and after becoming thus saturated is cooled so that condensation takes place on any particles remaining suspended. This sampler has not been widely used.

Impinger and Agar Samplers.

In impinger samplers a high velocity jet of the air being sampled strikes at close range against a collecting surface, which usually is solid agar culture medium. As the air stream is deflected by the collecting surface into turning a sharp right-angled corner at high speed, the suspended particles are centrifuged out, their momentum carrying them at a tangent straight on to the collecting surface. The efficiency of an impinger depends upon this centrifugal force and thus on the angular velocity with which the air stream is turned.

Pouchet (1860) developed an impinger sampler, the first "aeroscope", in which, by means of a water aspirator, measured volumes of air were drawn through a funnel into a small chamber where it impinged on an adhesive-coated slide held at 1 mm. from the ½-mm. diameter jet which formed the inner end of the funnel.

Hollaender and Dalla Valle (1939) designed the "funnel/

"funnel impinger", a simple device for sampling on to the whole surface of an agar culture plate. A Petri dish containing agar medium is placed on the bottom of a small airtight box. By aspiration from the box, air is caused to pass into the box at 1 cu. ft. per min. through an inverted funnel which is situated directly over the Petri dish so that its rim is within the dish rim and is less than 1 cm. from the agar surface. Comparing the "funnel impinger" with the "slit sampler", Bourdillon, Lidwell and Thomas (1941) found that the "funnel impinger" was efficient only for the larger bacteria-carrying particles; its overall efficiency for the infected particles naturally present in the air of a crowded room was 75%, for a coarse spray of Strept.salivarius in broth 45%, for a medium spray of the same 7%, and for a fine spray of Strept.salivarius in water only 1½%. Bourdillon and Lidwell (1948 b) state that, as a low velocity impinger, the "funnel impinger" is not efficient for particles smaller than 10 microns in diameter.

Du Buy and Crisp (1944) designed the 300-hole "sieve impinger". This resembles the "funnel impinger", except that the air intake is through 300 small (0.8 mm. diameter) holes in a brass plate held closely over the surface of the agar (e.g. at 2 mm. above it). Air is sampled usually at the rate of 1 cu. ft. per minute. The bacteria-carrying particles are deposited on the agar opposite the holes/

holes, but, as there are 300 of these, overcrowding is avoided. In parallel tests with the "funnel impinger", the 300-hole "sieve impinger" collected nearly twice as many bacteria-carrying particles from equal volumes of air.

Berry (1941) and Luckiesh, Taylor and Holladay (1946) have designed "electrostatic air samplers" in which air is passed over culture plates and the suspended particles are precipitated on to the plates by an electrostatic field.

The best known and most widely used of impinger and agar samplers are the "air centrifuge" of Wells (1933) and the "slit sampler" of Bourdillon, Lidwell and Lovelock (1941).

The "Air Centrifuge".

In the "air centrifuge" of Wells (1933), the air to be sampled is drawn at 1 to 2 cu. ft. per minute down a stationary central tube into the bottom of a larger tube which is lined with agar medium and is rotating at high speed (e.g. at 4000 r.p.m.); as the air escapes upwards through the rotating tube, it acquires the rotatory movement and the particles suspended in it are thereby centrifuged outwards on to the agar surface. The main disadvantages of this sampler are its low efficiency for the smallest bacteria-carrying particles and the inconvenience of preparing and examining the culture tubes.

Macdonald/

Macdonald (1940 b), making a comparison with a water-spray sampler supposed to be 98% efficient, found that the "air centrifuge" was 75% efficient for normal airborne bacteria.

Phelps and Buchbinder (1941) found that in comparison with the recovery by sedimentation for 8 hours (when 85% complete), the "air centrifuge" collected only 30 to 50% of the infected droplet-nuclei produced by fine spraying of a broth culture.

In comparison with the "slit sampler", Bourdillon, Lidwell and Thomas (1941) found that the "air centrifuge" collected from equal volumes of air only 72 to 76% of the bacteria-carrying particles occurring naturally in the air of a crowded room, 35 to 45% of bacteria sprayed finely from serum broth, and 5 to 6% of bacteria sprayed finely from distilled water. Bourdillon and Lidwell (1948 b) state that the "air centrifuge" is relatively inefficient for particles smaller than 10 microns.

The "Slit Sampler".

The "slit sampler" of Bourdillon, Lidwell and Thomas (1941), and its subsequent modifications, undoubtedly is by far the best of all instruments for the bacteriological examination of air. It is an impinger of maximum efficiency, even for the smallest bacteria-carrying particles. It is most convenient to use. In its original form it sampled 1 cu. ft. of air per minute on to a standard $3\frac{1}{2}$-inch diameter Petri dish/

dish containing agar medium. The "large slit sampler" of Bourdillon, Lidwell and Thomas (1948) samples at the rate of 20 cu. ft. per minute on to 6-inch diameter plates; this is for examination of air containing few bacteria. The timing arrangements described by Bourdillon, Lidwell and Schuster (1948) enable the taking over a short period of a continuous timed record of the minute-to-minute variations in the bacterial content of the air. The special "slit sampler" described by Schuster (1948) allows such a continuous record to be taken over a 12-hour period, automatically and without need for attention.

In the "slit sampler", the agar plate is contained in a small airtight chamber and lies on a platform which rotates at a known rate. Air from outside is drawn through a narrow intake-slit set at 2 millimeters above the agar surface, opposite a radius of the plate. The rate of air flow is controlled manometrically. The airborne particles are deposited on the strip of the agar plate which is under the intake-slit at the moment of their entry.

Bourdillon, Lidwell and Thomas (1941) developed various methods for determining the collection efficiency and the estimation efficiency of air samplers. They found that the collection efficiency of the "slit sampler" for the smallest bacteria-carrying particles, cocci sprayed finely from distilled water, was 94 to 97%, and that for larger bacteria-carrying particles, cocci sprayed coarsely from/

from serum broth or the infected particles of normal room air, it was 97 to 99.6%.

Du Buy, Hollaender and Lackey (1945) reported surprisingly low efficiency values for the "slit sampler", but Bourdillon and Lidwell (1948 a) have shown that their calibration tests were made without proper control of the air intake rate.

Liquid and Solid Collection Media, and Demonstration of Bacterial Clusters.

Du Buy and Hollaender (1945) compared samplers collecting on to solid agar medium (the "funnel impinger", the "sieve impinger", the "air centrifuge" and the "slit sampler") against samplers collecting into liquid broth (the "bead bubbler" and the "atomiser bubbler"). With air containing sprayed E.coli, the broth samplers gave bacterial counts which were 10 to 30 times higher than those given by the agar samplers. Du Buy and Hollaender explained this higher count as being due, not to greater collection efficiency on the part of the broth samplers, but to breaking up of the bacterial clusters on each particle when collected in the broth; the separated bacteria yield higher colony counts than are obtained with the agar samplers, when only one colony develops from the whole bacterial cluster on each airborne particle. The validity of this explanation was confirmed in two ways. Comparative tests were made for a spray of broth/

broth containing the dye uranine, instead of E.coli; an agar sampler, the "sieve impinger", collected half as much of the dye as did a broth sampler, the "bead bubbler", proving that the former had a much higher relative collection efficiency than was indicated by the bacterial counts. Higher bacterial counts were obtained by broth "settling dishes" than by agar "settling dishes" exposed in parallel, although the collection efficiency must have been the same in each.

In sampling the naturally infected air of crowded rooms, Wheeler and Jones (1942) obtained much higher (over 10-fold) bacterial counts with a broth sampler, the "bead bubbler", than with an agar sampler, the "air centrifuge", while, on the other hand, Bourdillon and Lidwell (1948 c) obtained slightly lower (80-90%) counts with a broth sampler, the Lemon "Folin-tube bubbler", than with an agar sampler, the "slit sampler".

Miles (1948) exposed in parallel broth "settling dishes" and agar "settling dishes" to the air of an animal house; the broth dishes gave counts which were $1\frac{1}{2}$ to $2\frac{1}{2}$ times greater than those given by the agar dishes, indicating that there had been some breaking up of bacterial clusters in the broth. Miles developed the "cascade method" for detecting and studying bacterial clusters collected on air-sampling plates. Molten agar at 43 to 45 deg.C. is poured down over the sloped surface of the plate before/

before incubation; the break-up of clusters is evidenced by the development of straight chains of identical colonies. By thus treating plates used to sample the air of occupied rooms, Miles observed chains of from 2 to 400 colonies (mainly 2 to 60) in the case of various micrococci, Gram-negative and Gram-positive non-sporing bacilli and Mucor.

The Hesse-Tube Method.

Hesse's method of sampling air, as practised by Carnelley, Haldane and Anderson (1887) and Carnelley and Foggie (1894), consists of drawing air slowly through a long narrow tube lined with nutrient gelatine. The bacteria-carrying particles settle on the walls of the tube and give rise to colonies on incubation. Although collecting by gravitation rather than by impingement, this simple method probably has an efficiency comparable to that of good impinger samplers, for all but the smallest bacteria-carrying particles.

(11) Experimental Infection of Air by Dust-Raising.

Experimentally, one of the easiest ways to produce heavy bacterial contamination of the air, is to raise up dust. In developing oil-impregnation methods for the laying of dust, van den Ende and his colleagues made observations on the extent to which air is infected by the raising of dust from floors and from bedclothes.

Van den Ende, Lush and Edward (1940) infected blankets by spraying with a serum-broth culture of haemolytic streptococcus (of group C usually). After drying, the blanket was hung in the centre of a 1000-cu. ft. room and given 5 to 20 strokes with a beater. Heavy infection of the air was produced; blood-agar "settling dishes" exposed for 10 minutes during and after the beating, collected from 100 to 700 infected particles. Infected blanket dust was allowed to settle on the linoleum-covered floors of two similar rooms, the floor of one having been treated with spindle oil. After the air had become free from infection, the floors were swept for 1 minute with a sterilised broom; heavy air infection was produced in the room with the unoiled floor; "settling dishes" exposed for 10 minutes during and after the sweeping gave on average 99 colonies in the room with the unoiled floor, and 0 colonies in the room with the oiled floor.

Van/

Van den Ende, Edward and Lush (1941) infected serge, wool-blanket and cotton-sheet strips by spraying with a serum-broth culture of group-C haemolytic streptococcus. Rubbing and beating of the strip liberated many infected dust particles into the air, as evidenced by the counts obtained on blood-agar "settling dishes" exposed above the strip. When, prior to infection, the cloth strips were treated with 10% liquid paraffin in white spirit and dried, the subsequent infection of the air was only about 10% as great as that given by the unoiled strips. Similar results were obtained with blankets infected naturally by use.

Hamburger, Puck, Hamburger and Johnson (1944) found that when a sheet or blanket from the bed of a scarlet-fever patient or convalescent was suspended from the ceiling and forcibly shaken, samples of air taken nearby with the Moulton "atomiser bubbler" sometimes yielded hundreds of haemolytic streptococci per 10 cu. ft. Sampling at later than 15 minutes after the shaking revealed that only a few of the streptococci remained airborne.

Bourdillon and Colebrook (1946) employed the "slit sampler" to observe the contamination of air with dustborne bacteria from used blankets, sheets, pillows, pyjamas and night-gowns; the shaking of one of these articles 3 or 4 times in a 3042-cu. ft. room at once infected the air to the extent of 27 to 565 bacteria/

bacteria-carrying particles per cu. ft. Ordinary body movements were found to infect the air with dust from the skin and clothing; when doctors or nurses wearing sterile gowns walked briskly round the room, sampling at 3 ft. above the floor revealed an increase in the number of bacteria-carrying particles in the air by 7.5 to 30 per cu. ft.

Dumbell, Lovelock and Lowbury (1948) employed the "slit sampler" to measure the amount of bacterial contamination of air caused by the shaking of soiled handkerchiefs. The handkerchiefs were 1-foot sided squares of Egyptian cotton; they were sterilised before issue; prior to the test they were used by a "normal" person for 2 days. The test was made in a 400-cu. ft. airtight room. An operator wearing only bathing trunks and a mask, and with wet skin and hair, imitated the actions of a person using his handkerchief; four times in succession he withdrew the handkerchief from its pot, shook it to unfold it, held it near his nose, and then replaced it in the pot. In ten tests the number of bacteria-carrying particles liberated into the air ranged between 1600 and 47,000, averaging about 15,000. Mechanical shaking liberated 5 times as many particles as hand shaking. The majority of the bacteria put into the air were commensal staphylococci originating from the nose and skin; in the case of nasal carriers, numerous Staph.aureus were among the bacteria liberated/

liberated. "Die-away" observations on the infected dust particles gave sedimentation rate values corresponding to an average particle diameter of about 20 microns. It was concluded that the use of the handkerchief is the most important single action, except bed-making, in contaminating the air with microorganisms from the respiratory tract.

Buchbinder, Soloway and Solotorovsky (1945) made "air centrifuge" observations in 7 vacated schoolrooms on the effect of sweeping the floor. On average, the number of bacteria-carrying particles per cu. ft. of air sampled on to blood agar, was 8.0 before sweeping, 26.2 just after sweeping, and 8.0 at 1 hour after sweeping.

(12) The Total Bacterial Content of the Air.

The flora of the air of occupied rooms, even when some pathogenic bacteria are present, consists predominantly of saprophytic and commensal species, including Staph.albus, Micrococci, diphtheroid bacilli, aerobic sporing bacilli, coliform bacilli, Streptomyces species and Fungi. Although the occurrence of these non-pathogenic microorganisms is not of any direct hygienic significance, many studies have been made of the extent and variations of the total bacterial content of the air, on the assumption that the factors which influence it, including population density, amount of movement and degree of ventilation, will also influence the extent of air infection with pathogenic bacteria.

The pioneer observations of Pasteur on the occurrence of putrefaction in flasks of broth receiving small portions of air, showed clearly that the bacteria are distributed in the air unequally as between different places.

Carnelley, Haldane and Anderson (1887), using the Hesse-tube method, examined the air in schools, dwelling houses and streets in Dundee. Air was drawn slowly through gelatine-lined tubes, 70 cm. long and 3.5 cm. wide, and these were incubated at room temperature for 3 to 4 weeks. The average number of bacteria-carrying particles in the air was found to be 1700 per cu. ft. in 1-room dwelling houses/

houses, as compared with 126 per cu. ft. in houses with 4 or more rooms, and 4300 per cu. ft. in schools ventilated naturally with fire-heating, as compared with 470 per cu. ft. for schools with habitual mechanical ventilation. Dust-raising was found to increase enormously the bacterial content of the air; in one case the stamping of feet raised it from 310 to 4200 per cu. ft., and in another case the making of beds in a hospital raised it from 79 to 790 per cu. ft. In the "open air" also, the colony counts were high, namely 472 per cu. ft. for the streets and 21 per cu. ft. for quiet places. The authors suggested that in houses and schools a standard of about 500 bacteria-carrying particles per cu. ft. should not be exceeded. It is to be noted that the bacterial counts obtained in this and other early studies were very much higher than the counts which have been obtained in recent studies made with more efficient air sampling devices; the probable explanation of this is that the prolonged incubation at room temperature adopted in the early studies, allowed growth of many saprophytic bacteria which would not grow in the short 37-deg.C. incubation now generally practised.

Carnelley and Foggie (1894) reported further similar observations on the air of schools.

Graham-Smith (1903) made observations of the microorganisms in the air of the House of Commons; he used/

used the sampling method of Frankland (1887), culturing the glass-wool filter plugs in gelatine at 20 deg.C. In the mechanically ventilated chamber during a debate when 300 to 400 members were present, microorganism counts ranging from 125 to 300 per cu. ft. were obtained in 19 samples, each of 4½ litres, taken at different positions. The average of the counts from 11 such experiments in the debating chamber was 165 per cu. ft. As the outside air gave counts of 36 to 120 per cu. ft., the air of the chamber was not thought to be especially heavily infected. In the committee, dining and smoking rooms, which were not mechanically ventilated, the air contained greater numbers of microorganisms; the average of the counts in 6 experiments was 910 per cu. ft. Microorganisms of species known to be pathogenic to man were not isolated from the air of the House of Commons on any occasion.

Forbes (1924), also using Frankland's method, examined the atmosphere of the London Underground Railways. In 5-litre samples cultured in agar at 37 deg.C., the average number of bacteria recovered was 113 per cu. ft. from the air of occupied carriages, 152 per cu. ft. from the air of crowded platforms, and 63 per cu. ft. from the outside air at street level. Samples cultured at 20 deg.C. gave bacterial counts which were almost double those obtained by culture at 37 deg.C. The bacterial content/

content of the underground air usually, but not invariably, increased and decreased with the passenger density. Pathogenic bacteria were not found on any occasion. The microorganisms present in the air were mainly saprophytic species, including Micrococci, B.subtilis, B.mesentericus, Sarcina, Staph.albus, B.xerosis, Mic.salivarius, Mic.cereus, Streptothrices and Fungi.

Wells and Wells (1936) reported an extensive series of observations made with the "air centrifuge", culturing on blood agar at 37 deg.C. Among many 10-cu. ft. samples the average number of bacteria-carrying particles in the air was 2.8 per cu. ft. for a modern air-conditioned theatre, 9.9 and 26.1 per cu. ft. for modern and old schools, 6.5 per cu. ft. for college classrooms and laboratories, 9.1 and 43.8 per cu. ft. for a lecture hall before and after a lecture, 72.8 per cu. ft. for children's clinics, 24.7 per cu. ft. for various hospital wards, 23.0 per cu. ft. for an operating room, 36.6 to 309.0 per cu. ft. for different rooms in a cotton textile mill, 11.2 to 36.2 per cu. ft. for rooms in a wool textile mill, and 1.1 to 2.5 per cu. ft. for out-of-door air in different seasons.

Torrey and Lake (1941) with the "air centrifuge" took several 20-cu. ft. samples of air in each of the 12 different departments of a large store. The average number of bacteria-carrying particles in the air/

air ranged from 2.9 per cu. ft. in the furniture department to 22.6 per cu. ft. in the general-merchandise department. The total bacterial count did not vary directly with the population density, but rather according to the type of merchandise handled; for instance, the bacterial count was high, 15.3 per cu. ft. in the rugs department, where the number of occupants was minimal, but where much dust was raised by the turning over of the rugs.

Buchbinder, Solowey and Solotorovsky (1945) reported an extensive investigation of the air of enclosed places in New York. During one year a total of 5000 samples of 10 cu. ft. of air were taken with the "air centrifuge", half on to blood agar and half on to plain agar. The overall average counts of airborne bacteria-carrying particles were 23.8 per cu. ft. in schools, 14.8 per cu. ft. in subways, 10.6 per cu. ft. in non-air-conditioned theatres, 2.5 per cu. ft. in air-conditioned theatres, 8.9 per cu. ft. in the streets, and 2.4 per cu. ft. in the park. Slightly higher counts were obtained on blood agar than on plain agar (25 to 50% higher). The amount of air infection varied with the population density, the amount of activity and the degree of ventilation. Lower counts were obtained in vacant school classrooms (on average, 12.6 and 9.7 per cu. ft. on blood agar and plain agar) than in occupied classrooms (27.0 and 21.1 per cu. ft.), and lower

counts/

counts were obtained in normally occupied subway cars (14.7 and 10.1 per cu. ft.) than in fully crowded subway cars (20.3 and 14.0 per cu. ft.). The population density was much greater in the fully crowded subway cars than in the occupied classrooms, and the lower bacterial contamination of the air in the former was attributed to better ventilation. The influence of ventilation was also exemplified in the markedly lower counts obtained for theatres with air-conditioning as compared with theatres without air-conditioning.

Bourdillon and Colebrook (1946), using the "slit sampler" with accurate timing arrangements, observed the minute-to-minute variations in the number of bacteria-carrying particles in the air of a burn dressing-room, and correlated these with concurrent activities. Air infection peaks, of 40 to 350 per cu. ft., corresponding to liberation of many dustborne bacteria into the air, were found to occur when the patient's blankets were gently disturbed, as in folding them down, when the bandages were cut, when the bandages and dressings were removed, when a woollen sock was pulled on to the patient's foot, when the patient waved his arm vigorously, and when the patient was lifted from a trolley on to a table; little air infection was caused when the patient was wheeled on his bed into the dressing-room. When the room was ventilated artificially with filtered air at the rate of about

10/

10 overturns per hour, the peak concentrations of air infection disappeared very rapidly, a low level being attained within 5 or 10 minutes, so that there was not any build-up of air infection. Without this artificial ventilation, the bacteria disappeared from the air only slowly (e.g. to half in 37 minutes) and tended to remain numerous until reinforced by a fresh arrival.

Colebrook and Cawston (1948), making "slit sampler" observations during one year, found that the number of bacteria-carrying particles in the air of surgical wards in a hospital was large, mainly 10 to 20 per cu. ft., on the street outside the hospital was smaller, 1.5 to 11.3 per cu. ft. (on average 4.8 per cu. ft.), and on the 75-feet high roof of the hospital was smallest, 0.38 to 14.6 per cu. ft. (on average, 2.0 per cu. ft. ). The roof air contained mainly Staph.albus, Micrococci, Bacillus species, Streptomycetes and Fungi: the only human pathogens found were Cl.welchii in moderate numbers and Staph.aureus in small numbers.

Bourdillon, McFarlan and Thomas (1948) examined the air of operating theatres with a "slit sampler". They found that with plenum ventilation, as at 7½ overturns per hour, the number of bacteria-carrying particles in the air tended to remain at a low level, 5 to 10 per cu. ft., except for large but temporary increases, to between 20 and 200 per cu. ft., which

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were caused by such occurrences as the entry and exit of patients, lifting of the patient between trolley and table, changing of the patient's position on the table, bandaging, changing plasters, and moving of the patient's blankets. With ventilation by an exhaust fan only, a much higher level of air infection was maintained, apparently due in considerable measure to the sucking-in of infected air from adjoining rooms.

Lidwell (1948), using the long duration "slit sampler", made continuous records of the air infection in the bedroom of a dwelling house during 12 hours overnight, and of that in the dining room during 12 hours in the daytime. In the bedroom when the occupants were asleep and in the dining room when it was unoccupied, only 1 to 5 bacteria-carrying particles were present per cu. ft. of air; large increases, of up to between 40 and 120, or more, per cu. ft., were closely correlated with movements of the occupants, as in drawing curtains, turning down the bed, changing the baby's napkins, dressing, making the bed, setting a meal, and sweeping the floor; disappearance of the bacteria added to the air on each occasion was rapid, for instance to 10% in 30 minutes.

Bourdillon, Lidwell, Lovelock and Raymond (1948) made "slit sampler" observations in a great variety of commercial and industrial premises. They found that the number of bacteria-carrying particles in the air ranged from 16 to 66 per cu. ft. in offices for typists/

typists and clerks, from 5 to 28 per cu. ft. in large engineering machine shops, from 0.5 to 320 per cu. ft. in workshops and foundries, from 30 to 220 per cu. ft. in crowded canteens, from 25 to 140 per cu. ft. in stores, and from 20 to 2160 per cu. ft. in hospital wards. The authors suggested a standard of 50 per cu. ft. as the upper limit to be considered hygienically satisfactory.

These various observations lead to the conclusion that the extent of the total bacterial content of the air in enclosed places varies directly with the population density and the amount of bodily movement and other dust-raising activity, and inversely with the amount of ventilation, when this is great.

(13) Strept.viridans as an Indicator of Contamination of the Air with Bacteria from the Respiratory Tract.

Gordon (1904) suggested that in air-hygiene studies the degree to which air in any place is subject to contamination with bacteria from the respiratory tract, may be gauged from counts of the Strept.brevis contained in it. Strept.brevis, a supposed variety of the Strept.viridans group, is a commensal bacterium which is present in large numbers in the saliva and throat secretions of all persons, and does not, as far as is known, originate from any other habitat; Gordon recovered Strept.brevis only occasionally and only in small numbers from out-door air in London, but commonly and in considerable numbers from the air of rooms in which a person had been speaking. Gordon (1906) proposed that, similarly, Staph.epidermidis albus might be observed as an indicator of contamination of air from the skin; he recovered this coccus from the air of a barber's shop and from the air of an operating theatre, but not from out-door air in London.

Wells and Wells (1936), reviving Gordon's test, proposed that the alpha-haemolytic streptococcus, Strept.viridans , recognised by its appearance on blood agar, should be counted as an indicator of nasopharyngeal contamination of air. They reported the results of 2000 air samples taken in New York; "alpha streptococci" were found in small numbers, namely/

namely between 0.06 and 1.33 per cu. ft. and comprising about 2% of the total flora, in the air of college classrooms, a lecture hall, public schools, a theatre, children's clinics, hospital wards and operating rooms.

Buchbinder, Solowey and Solotorovsky (1938) took 2517 samples of air with the "air centrifuge" from schools, subway cars, theatres, streets and a park in New York. Alpha haemolytic bacteria, presumed to be streptococci, were found in all types of location, the counts ranging from 0.03 per cu. ft. in the park to 0.15 per cu. ft. in the schools. A total of 1949 of these "streptococcus" strains were studied in detail and were classified by their fermentation reactions according to the Holman system: 58.4% as Strept.salivarius, 16.3% as Strept.mitis, 9.7% as Strept.equinus, 8.6% as Strept.ignavus, 4.4% as Strept.faecalis and 2.7% as Strept.nonhaemolyticus; it was concluded from this that the majority of the streptococci found in the air had originated from the nasopharynx (i.e. the salivarius, mitis and ignavus strains). Of all the 1949 alpha-haemolytic strains counted as "streptococci", only 39.6% showed the characteristic streptococcal "chain" morphology when examined microscopically in 18-hour broth subcultures. The remaining 60.4%, including some strains in all of the Holman classes, appeared as pairs, tetrads or irregular clusters of Gram-positive cocci. These

were/

were termed "putative streptococci", and were thought to be streptococci derived from the respiratory tract but altered in morphology as a result of their aerial environment.

Torrey and Lake (1941) used the "air centrifuge" to examine the air of a large department store. They identified the collected streptococci by their morphology in smears examined microscopically. In the different departments the number of streptococci in the air ranged from 0.008 to 0.09 per cu. ft. and comprised about 0.5% of the total flora. The streptococcus counts, unlike the total bacterial counts, seemed to bear some relation to the degree of crowding and to parallel closely the incidence of disabling colds among the store employees. Of 292 streptococcus strains isolated from the air, 40% were identified as Strept.salivarius, 22% as Strept.mitior, 24% as Strept.ignavus, 6.2% as Strept.equinus and 6.5% as Strept.faecalis.

Microscopical identification of streptococci recovered from the air is a laborious procedure, and identification by naked-eye observation of alpha-haemolytic zones surrounding the colonies on blood agar can not be regarded as satisfactory as long as the status of the "putative streptococci" remains in doubt. In the meantime, therefore, it seems proper to adopt an air-sanitation standard based on the "total bacterial count", as proposed by Bourdillon, Lidwell/

Lidwell, Lovelock and Raymond (1948), rather than a standard based on an "alpha-streptococcus count", as proposed by Wells and Wells (1936). In support of this decision may be quoted the finding by Buchbinder, Solowey and Solotorovsky (1945) that the "alpha streptococcus counts" paralleled closely the "total bacterial counts" (in the ratio of about 1 to 100).

(14) Pathogenic Bacteria in the Air of Enclosed Places

The air of rooms and other enclosed places which are occupied or visited by infected persons, usually is found to contain the specific pathogenic bacteria, but only in small numbers which seldom exceed 1% of the total air flora.

Haemolytic Streptococci in the Air.

Friedemann and Deicher (1926) and Vas (1926) are quoted by Hare (1940 b) as having demonstrated the presence of haemolytic streptococci in the air of scarlet-fever wards.

Cruickshank (1935), exposing blood-agar "settl-:ing dishes" for 30 minutes, recovered haemolytic streptococci from the air of burns wards occupied by patients having a high incidence of haemolytic streptococcal infection of their burns (11% on admiss-:ion rising to 50% after 2 days in hospital) and of their throats (6.5% on admission rising to 25.6% after 2 days).

White (1936) recovered haemolytic streptococci from the air of all of 27 single-bed puerperal fever wards; 14 of the 27 patients had haemolytic strep-:tococcal infection of the nose or throat as well as of the uterus. A pair of blood-agar "settling dishes" were exposed for 6 hours overnight, a second pair for 20 minutes during the morning toilet and a third pair for 20 minutes during sweeping; the 6-plate count of haemolytic streptococci ranged from 1 to 100, being in most cases between 5 and 50. The air/

air of similar wards housing patients not infected with the haemolytic streptococcus, yielded only 1 or 2 of the streptococci on the 6 plates, or none at all. By serological typing, it was shown that 39 out of 51 strains recovered from ward air were of the same type as the strain infecting the occupant of the ward. In two experiments in wards vacated by the occupant, in-:fection of the air with haemolytic streptococci was shown to be produced as a result of sweeping and bed-:making.

Brown and Allison (1937) examined the air in four large 22-bed scarlet-fever wards by exposing sets of 6 blood-agar "settling dishes" for 3-hour periods. On one day the numbers of Strept.pyogenes-carrying particles collected on the 6 plates in each of the four wards were 0, 2, 19 and 4 at night, 70, 158, 334 and 114 in the morning, and 18, 126, 228 and 92 in the afternoon. The exceptionally high level of air infection in the morning was attributed to the dust-raising caused by bed-making, sweeping of the floor and cleaning of the furniture. Of the haemolytic streptococcus strains recovered from the air, about 80% belonged to the serological types 1, 2 and 4, the prevalent nose and throat infecting types. In general, the "settling dishes" collected streptoc-:occus types originating from distantly-situated patients as often as types coming from nearby patients; this showed that the streptococci were distributed from the patient to the air of all parts of the ward.

Buchbinder/

Buchbinder, Solotorovsky and Solowey (1938), making numerous "air centrifuge" observations in schools, subways, theatres and streets in New York, recovered from the air a total of 52 strains of beta-haemolytic streptococci (47 from indoors and 5 from the streets). Of the 52 strains, 46 strains belonged to Lancefield's group A (including 4 street strains), 1 strain to group B, 3 to group C, 1 to group D and 1 to group G. Buchbinder, Solowey and Solotorovsky (1945) in a further report of this investigation stated that the 52 beta streptococci were isolated in samples yielding a total of 1949 alpha "streptococci", giving a ratio of 1 to 40.

Hart (1938a) reported observations made in 37 operating rooms in 33 hospitals in different parts of the U.S.A. During operations, between 1 and 16 "settling dishes" were exposed for 1-hour periods. Haemolytic streptococci were recovered in small numbers from the air of 9 of the 37 operating rooms.

MacDonald (1940 a) with the "air centrifuge" took 443 air samples totalling 4430 cu.ft. from 22 locations in the operating rooms, delivery rooms and corridors of a 600-bed general hospital during 7 months in the winter. The overall average degree of air infection with beta-haemolytic streptococcus particles was 0.002 per cu.ft., comprising 0.01% of the total bacterial content of the air. The haemolytic streptococci were found in the air of 2 out of the 10 operating rooms examined with the "air centrifuge"./

centrifuge".

MacDonald (1940 c) with the "air centrifuge" sampled a total of 320 cu.ft. of air in various locations in a maternity hospital during an epidemic of scarlet fever due to the type-3 haemolytic strep-:tococcus. The average degree of air infection with haemolytic streptococci was 0.013 per cu.ft., com-:prising 0.06% of the total bacterial content of the air.

Cruickshank and Muir (1940) studied an outbreak of haemolytic streptococcal throat infection affect-:ing 6 influenza convalescents confined to bed in a small ward. On the fifth and sixth days after admission, 4 of the convalescents developed pharyn-:gitis with fever, and subsequent swabbing showed that all 6 were infected with the type-1 haemolytic streptococcus, 2 being healthy carriers. Infection must have been airborne between the convalescents, since confinement to bed precluded contact, the nursing and domestic staff all yielded negative nose and throat cultures, and infection did not spread to 5 influenza convalescents in another ward. Three blood-agar "settling dishes" exposed for 2 hours during bed-making, sweeping and dusting, gave 90 colonies of the type-1 haemolytic streptococcus.

Miles and his colleagues (1940) exposed "settling dishes" on the beds of patients during dressing of their infected wounds, and in the case of 2 out of 14 patients collected 3 and 2 colonies of Strept. pyogenes./

Strept.pyogenes. They did not recover Strept.pyogenes from the air on any of 154 plates exposed in the centre of a ward full of wounded soldiers.

Bourdillon, Lidwell and Thomas (1941) used the "slit sampler" with gentian-violet blood-agar plates to examine the air of a crowded canteen, and recovered beta-haemolytic streptococci in about 50% of the samples, in numbers up to 0.3 per cu.ft.

Cruickshank (1941), by exposing during each successive hour two 4-inch diameter blood-agar "settling dishes", examined the course of air infec-:tion during one day in the single-bed ward of a boy who was convalescent from scarlet fever due to the type-2 Strept.pyogenes. The 2-plate-hour count of this streptococcus was practically zero during the night and fluctuated during the day from a general low level of between 10 and 20 to peaks of between 50 and 200 corresponding to the times of toilet, sweeping of the floor, bedmaking and dusting. This correspondence indicated clearly that the strepto-:coccal air infection was mainly dustborne.

Glass (1941) recovered a few group-A and group-C streptococci on "settling dishes" exposed for several hours in a single-bed puerperal fever ward occupied concurrently by a patient with a group-C streptococcus infection and occupied previously by a patient with group-A streptococcus infection.

Van den Ende and Spooner (1941) in a surgical ward tested the effect of dust-suppression by oil-treatment/

oil-treatment of the floor and bedclothes in reducing the total bacterial content of the air as measured by "settling dishes". Oil-treatment resulted in a general reduction of the bacterial content of the air by about 50% and in a flattening of the peaks corres-:ponding to sweeping and bedmaking. A few group-A haemolytic streptococci were recovered from the ward air prior to the oiling.

Thomas and van den Ende (1941), using the "slit sampler" with 1 in 500,000 gentian-violet blood-agar plates, observed the amount of air infection in Ear, Nose and Throat Wards. They found haemolytic streptococci to be present in the air of every ward having one or more patients with haemolytic strepto-:coccal infection of the upper respiratory tract; for instance, in a ward with 9 cases of tonsillitis, the airborne particles carrying haemolytic strepto-:cocci regularly numbered 3 to 5 per cu.ft., and in-:creased at the times of bedmaking, meal-serving and other similar activities. In single-bed cubicles, bedmaking increased the number of haemolytic streptococcus particles in the air to between 75 and 100 per cu.ft., and even to 187 per cu.ft.. Oil-treatment of the bedclothes in a 16-bed ward resulted in a 93% reduction in the total bacterial content of the air during bedmaking, as compared with a control 10-bed ward (averages of 1.5 per cu.ft. and 19.4 per cu.ft. on gentian-violet blood-agar). In the cubicle of a tonsillitis patient, oiling of the floor and bedclothes reduced the haemolytic-streptococcus air infection/

infection by about 90%, thereby proving that this was mainly dustborne in origin.

Spooner (1941) made 109 fifteen-minute blood-agar "settling dish" observations of air infection in a plastic-surgery ward. Strept.pyogenes-carrying particles were found to be falling from the ward air at the rate of about 6 per sq.ft. per hour. Quarter of the cases admitted to the ward acquired infection of their wound with Strept.pyogenes during their stay in the ward.

Brooks, Wilson and Blackfan (1942) recovered haemolytic streptococci from the air on to blood-agar "settling dishes" at 11 out of 56 examinations of certain wards in an infants' hospital, but only at 1 out of 56 examinations of other wards which were subjected to ultra-violet irradiation for the purpose of disinfecting the air.

Wheeler and Jones (1942) used blood-agar "settl-:ing dishes" and also the "air centrifuge" to make weekly observations of air infection in a rheumatic fever hospital. Haemolytic streptococci were recovered from 2.2% of 45 air samples from wards with ultra-violet irradiation, and from 5.6% of 689 air samples from non-irradiated wards; the haemolytic-streptococcus carrier rates were similar in the irradiated (53.5%) and non-irradiated wards (59.6%). Of 107 haemolytic-streptococcus strains recovered from the air, 94.4% belonged to group A; the frequency of the different Griffith's types among the air strains corresponded to their frequency among the/

the throat-carrier strains.

Colebrook, Gibson and Todd (1944) exposed blood-agar "settling dishes" for 1 or 2 hours during morn-:ing dressings in the centre of a 10-bed burns ward. Haemolytic streptococci were collected in small numbers on 5 of 52 plates exposed on 26 days.

Wright, Cruickshank and Gunn (1944) at 7 to 14-day intervals during a 12-week period used the "slit sampler" with gentian-violet blood-agar plates to examine 50 cu.ft. of air during bedmaking and 30 cu. ft. during sweeping of the floor in a measles ward in which there was a high incidence of secondary haemolytic streptococcal throat infection. The number of haemolytic streptococcus particles found in the air on the different occasions was 2.1, 5.3, 7.0, 2.9, 2.6, 1.9, 4.4 and 0.8 per cu.ft. (averaging 3.4 per cu.ft.) during bedmaking, and 1.2, 1.1, 1.7, 0.3 and 0.3 per cu.ft. (averaging 0.9 per cu.ft.) during sweeping; the haemolytic streptococci com-:prised about 0.1% of the total bacterial content of the air as measured by parallel sampling on ordinary blood-agar. In another similar ward, the floor was treated with spindle oil, and the bedclothes, includ-:ing blankets, sheets, counterpanes, pillow-slips, patient's garments, towels, gowns and curtains, were treated with technical white oil during laundering; in this oil-treated ward, the number of haemolytic streptococcus particles in the air on the different occasions was 0.14, 0.20, 0.04, 0.04, 0.00, 0.00 and 0.00/

0.00 per cu.ft. (averaging 0.06 per cu.ft.) during bedmaking, and 0.03, 0.00, 0.00, 0.00 and 0.00 per cu.ft. (averaging 0.006 per cu.ft.) during sweeping. The two wards had shown similar degrees of air in-:fection prior to the test. Thus, the oil-treatment had reduced the haemolytic-streptococcus air in-:fection by 98% during bedmaking and by 99% during sweeping. This affords further evidence that natural haemolytic streptococcus air infection is mainly dustborne in origin.

Hamburger, Puck, Hamburger and Johnson (1944) used the Moulton "atomiser bubbler" to examine the air of scarlet-fever, measles and respiratory-disease wards in which cross-infections with haemolytic streptococci were occurring and many of the patients were throat carriers. The air usually contained between 0 and 1 per cu.ft. of haemolytic streptococci, but on certain occasions as many as 3.6, 4.6 and 10.4 per cu.ft.. The highest air infection levels occurred almost exclusively at times when the floor was being swept or the bedlinen changed. Heavy air infection was often localised in "pockets"; high counts were often obtained in a sample taken near the site of sweeping or bed-changing, while low counts were obtained in a sample taken at the other side of the ward either just before or just afterwards. The haemolytic streptococci comprised only about 0.2% of all the bacteria in the air. In the case of two wards examined every few days for several weeks, the air/

air haemolytic-streptococcus counts were found to parallel closely the total bacterial counts in their day-to-day variations. This correspondence suggested that the haemolytic streptococci entered the air in the same way as the majority of the air bacteria, and thus, since the majority were dust saprophytes, that they entered the air by the stirring-up of ward dust.

Hamburger, Green and Hamburger (1945 a), using gentian-violet blood-agar "settling dishes", recovered 35 times as many haemolytic streptococci from the air of a ward containing patients carrying the strepto-:coccus in both nose and throat, as from the air of a ward containing patients carrying the streptococcus in the throat only.

Colebrook and Ross (1947) recovered a small number of type-1 haemolytic streptococci from the air of a burn dressing-room. The evidence suggested that the streptococci originated from a small scabbed-over lesion on the elbow of the surgeon working in the dressing-room.

Begg, Smellie and Wright (1947) used the "slit sampler" with gentian-violet blood-agar plates to make 17 weekly observations in a measles ward. Of the 186 patients nursed in this ward during the per-:iod, 19.7% carried haemolytic streptococcus in the nose or throat on admission, 12.4% became cross-infected with haemolytic streptococcus while in the ward, and 4.8% were heavy nasal carriers of haemolytic streptococcus/

streptococcus while in the ward; 18.8% of the 32 nurses and domestic servants of the ward carried the haemolytic streptococcus in the nose or throat. In 100-cu.ft. air samples taken during bedmaking on the 17 occasions, the haemolytic streptococcus particles numbered between 0.00 and 1.80 per cu.ft. and averaged 0.29 per cu.ft., comprising 0.12% of all bacteria-carrying particles in the air as counted in samples taken on ordinary blood-agar. In 100-cu.ft. air samples taken during sweeping, the haemolytic strep-:tococcus particles numbered between 0.00 and 1.50 per cu.ft., and averaged 0.14 per cu.ft., compris-:ing 0.10% of all bacteria-carrying particles in the air. Of 81 strains of haemolytic streptococci from the air, 79 belonged to group A, 1 to group C and 1 to group G. Parallel observations were made in a similar measles ward with a similar incidence of haemolytic streptococcus infection among the patients and nursing staff, but with the floor and bedclothes treated with oil; the average number of haemolytic streptococcus particles in the air during bedmaking was 0.015 per cu.ft., and during sweeping 0.0059 per cu.ft., representing reductions of 95% and 96% as compared with the unoiled ward. That dust-suppress-:ion by oiling should so greatly reduce the haemolytic-streptococcus content of the air, affords clear evidence that the latter is mainly dustborne in origin.

Diphtheria Bacilli in the Air.

Crosbie/

Crosbie and Wright (1941) used 3-inch diameter "settling dishes" with Hoyle's medium to examine the air of diphtheria wards. In a ward with 20 to 25 patients, the number of diphtheria-bacillus particles collected on 6 dishes exposed each for 1 hour during and after sweeping of the floor was 24, 10 and 15, on three occasions; the diphtheria-bacillus colonies comprised 2.4% of all colonies developing on the Hoyle culture dishes. After oiling of the floor the counts on four occasions were only 3, 1, 1 and 1, representing a reduction of over 90%. A few diphtheria bacilli were also recovered from the air of this ward at times when no sweeping was being done, once during a quiet period and once during bedmaking. In the cubicle of one patient, in which the floor had just been disinfected by mopping with a 50% solution of phenols, a few diphtheria bacilli were recovered from the air on two occasions during bedmaking. This investigation provides evidence that air in-:fection with the diphtheria bacillus is mainly dust-:borne in origin.

Meningococci in the Air.

Eagleton (1919) exposed "settling dishes" for $8\frac{1}{2}$ hours overnight in a sleeping hut occupied by soldiers, including some who carried the meningococcus in the nasopharynx. Meningococci, identified by agglutination tests, were recovered on 1 out of 13 dishes in one experiment, and on 1 out of 6 dishes in another experiment.

Staph.aureus in the Air/.

Staph.aureus in the Air.

Hart and his colleagues have investigated the high incidence of unexplained infection of "clean operation" wounds with Staph.aureus, and have att-:ributed these infections to settlement of Staph. aureus from the air of the operating room on to the wound or the surgical instruments and supplies (Hart, 1937, 1938 a, 1938 b, 1941, 1942; Hart and Schiebel, 1939; Hart and Upchurch, 1941) Hart (1937) examined the air of an operating room in which the strictest aseptic precautions were observed, and found usually from 6 to 78 colonies of haemolytic Staph.aureus per "settling dish" per hour. The operating-room person-:nel, who included up to 78% of nose and throat carriers of the staphylococcus, appeared to be the source of air infection. Hart (1938 a) reported ob-:servations made in 37 operating rooms in 33 hospitals in different parts of the U.S.A.. During operations, between 1 and 16 "settling dishes" were exposed for 1-hour periods. Haemolytic Staph.aureus were re-:covered in small or moderately large numbers from the air of 27 of the 37 operating rooms (e.g. 1 to 26 colonies per dish per hour, comprising about 10% of all colonies on the dish). Hart and Schiebel (1939) during one year made weekly examinations of the air of an operating room by the exposure of blood-agar "settling-dishes" for periods of 1 hour while the room was occupied; from 10 to 100 colonies of all kinds were obtained per dish per hour, and usually from 1 to 10 colonies of haemolytic Staph. aureus/.

Staph.aureus.

MacDonald (1940 a) used the "air centrifuge" to take 443 samples comprising a total of 4430 cu.ft. of air from 22 locations in the operating rooms, delivery rooms and corridors of a hospital. The overall average recovery of particles carrying haemolytic Staph.aureus was 0.35 per cu.ft., comprising 1.5% of all the bacteria-carrying particles recovered. Staph.aureus was found in the air of 8 out of 9 operating rooms.

Miles and his colleagues (1940) obtained a total of 12 colonies of Staph.aureus on 154 "settling dishes" exposed in the centre of a ward full of wounded soldiers.

Brooks, Wilson and Blackfan (1942) recovered Staph.aureus on 55 out of 56 blood-agar "settling dishes" exposed on different occasions in certain wards of an infants' hospital, and on 53 out of 56 "settling dishes" exposed in other wards which were subjected to ultra-violet irradiation for the purpose of disinfecting the air.

Bourdillon and Colebrook (1946) recovered Staph.pyogenes aureus from the air of a burns dressing-room more frequently than any other path-:ogenic bacterium, namely on 47 out of 68 "settling dishes" exposed each for 1 to 3 hours during dressings. "Slit sampler" observations revealed airborne Staph. aureus-particles numbering from 20 to 60 per cu.ft. during the dressing of two burn cases infected with this/

this staphylococcus; following departure of the second of these patients, the Staph.aureus air in-:fection remained above 20 per cu.ft. for more than 25 minutes (artificial ventilation was not being employed at this time).

Allison and Hobbs (1947) recovered small numbers of Staph.aureus from the air on blood-agar "settling dishes" exposed on two occasions in a nursery during outbreaks of pemphigus neonatorum; at these times, 87.5% and 61.1% of the healthy infants, and 66.7% and 68.8% of the nursery staff carried Staph.pyogenes in the nose.

Pneumococcus and H. influenzae in the Air.

Brooks, Wilson and Blackfan (1942) exposed blood-agar "settling dishes" in wards of an infants' hos-:pital. They recovered pneumococcus on 1 out of 56 dishes in ultra-violet irradiated wards and on 12 out of 56 dishes in non-irradiated wards, and H. influenzae on 1 out of 56 dishes in the irradiated wards and 0 out of 56 dishes in the non-irradiated wards.

Plague Bacillus in the Air.

Wu Lien Teh, Chun Wing Han and Pollitzer (1922) in 5 experiments did not recover P.pestis on any of 3 agar "settling dishes" exposed for ½ to 1 hour in the sickrooms of pneumonic-plague patients. In 2 out of 7 experiments, plague was contracted by rabbits or guinea pigs exposed for ½ to 96 hours in buckets on the floors of such sickrooms; these in-:fections/

infections were considered to be airborne.

Cl. welchii in the Air.

Buxton and Allen (1921) recovered Cl.welchii from the air of a stable on to "settling dishes".

Colebrook and Cawston (1948) with the "slit sampler" recovered Cl.welchii fairly commonly from the open air above the roof of a hospital.

Monilia albicans in the Air.

Ludlam and Henderson (1942) exposed Sabouraud-agar "settling dishes" for 4-hour periods to the aid of nurseries and changing rooms of a maternity hospital having a high incidence of neonatal thrush. Monilia albicans, a single colony, was recovered on only 1 out of 23 dishes.

Mechanism of Infection of Air with Pathogenic Bacteria.

The reported observations afford strong evidence that naturally-occurring air infection with haemolytic streptococci and diphtheria bacilli is mainly dustborne in origin; very probably, air in-:fection with other pathogenic bacteria is produced in the same way.

White (1936), Brown and Allison (1937), Thomas and van den Ende (1941), Cruickshank (1941) and Hamburger, Puck, Hamburger and Johnson (1944) found that the number of haemolytic streptococci in the air was high at the times of bedmaking, dusting, cleaning, sweeping, serving of meals, toilet and other dust-raising activities, and was low at "quiet" times. Crosbie and Wright (1941) found similarly that diph-:theria/

diphtheria bacilli were most numerous in the air at the time of sweeping of the floor.

Hamburger, Puck, Hamburger and Johnson (1944) found that the number of haemolytic streptococci in the air varied from day to day in a way closely paralleling the number of saprophytic "dust bacteria".

Thomas and van den Ende (1941), Wright, Cruick-:shank and Gunn (1944) and Begg, Smellie and Wright (1947) found that the number of haemolytic strep-:tococci in the air was very greatly reduced by oil-:ing methods of dust-suppression. Crosbie and Wright (1941) found similarly that oiling greatly reduced the amount of air infection with diphtheria bacilli.

(15) Mode of Entry of Bacteria into Respiratory Tract.

Probably the main modes of entry of pathogenic bacteria into the respiratory tract are: (1) by contact transfer into the mouth on fingers, eating utensils, food and milk, and (2) by inhalation into the nose on airborne particles. Certainly it is possible for bacteria to be transferred to the nasal mucous membrane by contact, as with the fingers in picking the nose, but the fingers and other objects are put into the nose less deeply and less commonly than into the mouth. Airborne bacteria may be inhaled into the mouth as well as into the nose, but mouth-breathing is less common than nose-breathing; in as much as the mouth filters the air less efficiently than the nose, infection by inhalation through the mouth may be important in the case of the primary lung infections.

Introduction of Bacteria into the Mouth.

The occurrence of food-borne and milk-borne outbreaks of scarlet fever and diphtheria is proof that the throat may become infected via the mouth, that ingested bacteria may reach and invade the tonsillar and pharyngeal membranes. However, large numbers of the infecting bacteria may be introduced in the food or milk, and it can not be taken for granted that a small dose of the bacteria also would infect by the oral route.

Experimental observations have revealed that the majority/

majority of bacteria introduced into the mouth either succumb to the antibacterial action of the secretions, or are swept in the salivary flow down the oesophagus into the stomach where they are killed; only a small minority of the bacteria entering the mouth can reach and attach themselves to the mucous membranes of the tonsils and pharynx, which alone provide a suitable habitat for colonisation.

Sanarelli (1891) found that streptococci and staphylococci were destroyed rapidly by freshly filtered saliva in vitro; pneumococci were not killed, but grew readily in saliva.

Miller (1903) is quoted by Appleton, Klein and Palmer (1938) as finding that B.prodigiosus disappeared from the mouth within 6 hours after rinsing of the mouth with a suspension of about 2,000,000 of these bacilli.

Barnes (1907-09) is quoted by Bloomfield (1919) as having found that saliva did not have any bactericidal action on streptococci or pneumococci.

Gordon (1916) found that growth of meningococcus was inhibited by saliva, apparently by an antibiotic action of the normal salivary flora since a young broth culture of this flora was equally effective.

Bloomfield (1919) inoculated 6 persons on the tongue with solid masses of Sarcina lutea culture. Scrapings from the mucosa were taken at intervals and examined/

examined by culture. The sarcinae were completely eliminated from the tongue within 1 hour in 4 persons and within 24 hours in the other 2 persons. Cultures from the pharynx showed that in 2 persons a few living sarcinae had reached this region within 10 minutes, but that none were present at 1 and 24 hours. Elimination appeared to be due to killing rather than to backwards flushing out of the mouth. When incubated *in vitro* with saliva, sarcinae were killed within 1 to 2 hours; sarcinae incubated with a suspension of mouth commensal bacteria were not killed.

Bloomfield (1920 a) in similar experiments studied the fate of *E.coli* and *Staph.albus* after their introduction into the mouth. A loopful of *E.coli* was placed on the tip of the tongue of 4 persons who did not feed during the following two hours. At 10 minutes and at 2 hours large numbers of living *E.coli* were found on the tongue and in the pharynx of all 4 persons; at 24 hours a few were found on the tongue and in the pharynx of only 1 person; at 48 hours none were found in any case. Similarly, 3 persons were inoculated on the tongue with *Staph.albus*. At 10 minutes and at 2 hours large numbers of living *Staph.albus* were found on the tongue and in the pharynx of all 3 persons; at 24 hours a few were found on the tongue and in the pharynx of 2 persons; at 48 hours a few were found on the tongue of 1 person only. Elimination of *E.coli* and/

and Staph.albus was much slower than elimination of Sarcina lutea. The former bacteria differed from the latter in that they were not susceptible to the bactericidal action of saliva; they were not killed when incubated in saliva for 24 hours. Apparently the elimination of E.coli and Staph.albus was due entirely to mechanical flushing by the salivary flow. It was observed that kieselguhr placed on the tongue was eliminated at least as rapidly as these bacteria; the kieselguhr could not be detected microscopically in scrapings taken at 2 hours after inoculation.

Bloomfield (1920 b) made similar observations with 3 strains of H.influenzae. He inoculated 4 persons on the tongue with a loopful of solid culture. At 10 minutes a large number of living H.influenzae were found on the tongue and pharynx of all 4 persons; at 2 hours a few were found on the tongue of all 4 persons and in the pharynx of 3 persons; at 24 hours a few were found on the tongue and pharynx of only 1 person; at 48 hours none were found in any case. H.influenzae was eliminated to some extent by mechanical flushing and to some extent by an antibacterial action of the saliva; when large numbers of the bacilli were incubated in vitro with fresh saliva, many remained alive for 2 hours, but all were killed within 24 hours.

Bloomfield (1920 c) made similar experiments with 3 strains of Friedlander's bacillus (K.pneumoniae).

He/

He inoculated 9 persons on the tip of the tongue with a loopful of solid culture. At 10 minutes many living bacilli were found on the tongue and pharynx of all 9 persons; at 2 hours many or few bacilli were found on the tongue of 8 persons and in the pharynx of 8 persons; at 24 hours a few bacilli were found on the tongue of only 2 persons and in the pharynx of only 1 person; at 72 hours living bacilli were not found in any case. Elimination seemed to be due entirely to mechanical flushing; when incubated in vitro with saliva, the bacillus multiplied and survived in large numbers for at least 2 days.

Bloomfield and Huck (1920) found that 102 saliva samples from 52 healthy persons ranged in pH from 6.0 to 7.3, four-fifths being between 6.6 and 7.1. The bactericidal action of saliva thus can not be due to an unfavorable reaction.

Bloomfield (1922 b) inoculated healthy persons on different parts of the mouth with solid masses of Sarcina lutea culture. At 10 minutes later all areas of the mouth were examined by culture; it was found that the sarcinae had not spread widely throughout the mouth, but only in a backwards direction towards the root of the tongue. After inoculation of the tip of the tongue, large numbers of living sarcinae were found on this part and on the tongue posteriorly, but few or none were found on the soft palate, the tonsils and the posterior/

posterior pharyngeal wall. After inoculation of the posterior pharyngeal wall, few living sarcinae reached the tonsils and none reached the tongue or other parts of the mouth. Similar results were obtained even when a large drink of water was taken between inoculation and the taking of specimens; the bacteria were not dislodged and distributed widely by drinking. When a heavy suspension of sarcinae was taken into the mouth and swallowed, a large number adhered to the tongue, but few to the tonsils, palate or posterior pharyngeal wall. These observations of Bloomfield confirmed his earlier observations concerning the elimination of carbon particles from the mouth (quoted by Wilson and Miles, 1946); carbon particles put into the mouth were seen to be removed in an orderly manner, being swept from the site of inoculation backwards in the salivary stream over the base of the tongue, without dissemination in other directions and without deposition on the tonsils, the removal being completed within 15 to 30 minutes.

Van der Reis (1921) is quoted by Appleton (1944) as having found that the eating of a meal usually resulted in complete elimination from the mouth of B.prodigiosus which had been introduced by spraying.

Many investigators have found that the diphtheria bacillus is inhibited or killed by saliva. Bézi (1932) found that the saliva of 3 out of 9 persons moderated

the virulence of diphtheria bacilli; the bacilli were mixed with filtered saliva and tested by intradermal injection of guinea pigs. Dold and Weigmann (1934) and Dold (1935) by *in vitro* experiments with plate subcultures proved conclusively that saliva is inhibitory or bactericidal towards the diphtheria bacillus. Weigmann and Noeske (1937) found that the inhibitory agent was removed by filtration, while Casassa (1937) found that it passed both Seitz and Berkefeld filters. Weigmann and Holzl (1940) and Holzl (1941) found that certain strains of the salivary commensal streptococci were antagonistic to the diphtheria bacillus; however, they thought that these commensals could not be mainly responsible for the inhibitory action of saliva, since they were effective only in very large numbers. Thompson and Shibuya (1946) found that pure cultures of the *Strept.mitis* type of viridans streptococci isolated from saliva inhibited the growth of diphtheria bacilli in the same manner as fresh saliva; they found that the inhibitory action of saliva could not be demonstrated after the streptococci had been removed from it by filtration, centrifugation, heat or the bactericidal effect of copper.

Appleton (1944) has reviewed various other observations of the antibacterial action of saliva for pathogenic bacteria. The lysozyme and thiocyanate contents/

contents of saliva may exert important effects. Kanter and Appleton (1940) observed that *in vitro* saliva exerted a bactericidal action against the tubercle bacillus.

Hood and Arnold (1937) found that *B.prodigiosus* was eliminated from the mouth on average within 2⅓ hours after its inoculation, and always within 5 hours.

Appleton, Klein and Palmer (1938) with 3 healthy subjects made 590 experiments in which 0.5 ml. of a *B.prodigiosus* suspension was rinsed through the mouth, and, after a predetermined interval in which eating, drinking, spitting and smoking were avoided, a single test was made for the presence or absence of the bacillus. For the 3 subjects the times of 50%-probability of complete elimination were 6¾, 9 and 15 hours when about 1,000,000 bacilli were inoculated, and 1¼, 1¼ and 3 hours when about 5000 bacilli were inoculated. Reporting on a continuation of this study, Appleton (1944) recorded that elimination was greatly hastened by increase of salivation following the taking of pilocarpine or the chewing of paraffin, the rate of elimination being increased by 3 to 4 fold; this suggested that the elimination was brought about mainly through mechanical flushing by the salivary flow.

Bloomfield (1919; 1920 a,b,c), in parallel with his experiments on inoculation of the tongue, made experiments in which the various bacteria were put into the tonsillar crypts of the healthy test subjects.

He/

He found that the inoculated bacteria were eliminated from the tonsillar crypts almost as rapidly as from the tip of the tongue; Sarcina lutea disappeared within 1 hour in 3 out of 4 persons, E.coli within 48 hours in 2 out of 3 persons, Staph.albus within 48 hours in 2 out of 3 persons, H.influenzae within 24 hours in all of 3 persons, and Friedlander's bacillus within 48 hours in 2 out of 3 persons. With these commensal and potentially pathogenic bacteria, permanent colonisation did not in any case follow artificial inoculation of the tonsillar crypts; in all cases the tonsillar membrane was able to free itself of the bacteria within 1 hour to 3 days. Thus it is clear that infection does not follow automatically the introduction of a potentially pathogenic bacterium to the tonsil. Presumably a combination of low resistance on the part of the host and high invasiveness on the part of the inoculated bacterium is necessary before colonisation and infection can take place.

The occurrence of circumstances favorable to the development of clinical infection or 'permanent' carriage, is apparent in the successful attempts at experimental inoculation of the human throat which have been reported by some investigators. Guthrie, Marshall and Moss (1921) swabbed a culture of virulent diphtheria bacillus on to the tonsils and posterior pharyngeal wall of each of 8 healthy adults who were not carrying the/

the diphtheria bacillus previously. Subsequent making of cultures from the throat revealed that 7 of the 8 persons became infected with the diphtheria bacillus and continued to carry the bacillus for 4 weeks or more. Of the 4 persons who initially were Schick-negative, 3 became "healthy carriers". Of the 4 persons who initially were Schick-positive, all 4 developed severe clinical diphtheria after the short incubation period of 1 day, and on recovery became "convalescent carriers". Bloomfield (1922 c) quoted Cecil and Steffen (1921) as having inoculated the throats of human volunteers with virulent strains of H.influenzae and having found the inoculated strains, identified by agglutination tests, persisting for periods which ranged from 6 hours to 2 months among the different subjects. Scarlet fever has been produced experimentally in man by inoculation of the throat with a culture of Strept.pyogenes; of 10 volunteers thus inoculated by Dick and Dick (1923), 2 contracted scarlet fever and 1 contracted febrile tonsillitis (see Wilson and Miles, 1946).

Inhalation of Airborne Bacteria into the Nose.

Hatch (1942) has reviewed the available information as to the locations within the respiratory tract on to which are deposited the airborne particles inhaled in normal nose-breathing. The airborne particles larger than about 5 microns in diameter, which probably include the/

the majority of the particles carrying pathogenic bacteria, are collected mainly in the nose; these large particles are removed from the inhaled air mainly by impingement on the air-passage wall at points where they are centrifuged out of a fast air-stream changing its direction; this occurs especially in the region just within the anterior opening of the nasal cavity, since this is the narrowest part of the respiratory tract and has the greatest air velocities. On the other hand, particles smaller than 5 microns in diameter tend to be inhaled deeply (only 15% of 1-micron particles are retained in the nose) and so reach the lung alveoli where, because the air velocity is minimal, they are deposited by sedimentation on the alveolar walls. Practically 100% of the inhaled particles larger than 5 microns are retained somewhere within the respiratory tract, but a proportion of the smaller inhaled particles, 80% in the case of those under 0.3 microns, are expelled again in the expired breath (Brown, 1931, and van Wijh and Patterson, 1940, quoted from Hatch, 1942). Rooks (1939) in tests with 7 subjects measured the filtering efficiency of the nose for bacteria-carrying droplets and droplet-nuclei; he found an efficiency of 88% for concurrently sprayed broth culture, of 85% for 6-seconds settled spray, and of 62% for 10-minutes settled spray.

The mucous membrane of the nasal cavity usually does/

does not provide a favorable habitat for colonisation by pathogenic bacteria. Upper respiratory tract infection usually is confined to the nasopharynx, oropharynx and tonsils; apparently the bacteria must reach these regions before colonisation can begin. A few of the inhaled bacteria may be deposited directly on the walls of the nasopharynx and oropharynx, but the majority will be deposited on the walls of the nasal cavity; these latter will be carried backwards to the nasopharynx by the flow of secretion over the ciliated membrane of the nasal cavity, and if they survive the bactericidal action of the nasal secretion they may then infect the nasopharynx.

The eliminative mechanism of the nasal cavity was first studied by Thomson and Hewlett (1896). These investigators found that the healthy nasal membrane is practically sterile. They placed a loopful of B.prodigiosus culture on the membrane of the nasal septum. By subsequent cultural examinations they found that the number of bacilli surviving at the site of inoculation was reduced to 25% in 1 hour and to 0% in 2 hours. Elimination appeared to be due to killing rather than to transportation, since cultures made from surrounding areas did not reveal many living bacilli.

Calvino (1899) is quoted by Bloomfield (1919) as having found that the nasal mucous membrane is an unfavorable medium for some types of bacteria.

Bloomfield/

Bloomfield (1919) inoculated 5 persons on the membrane of the nasal septum with masses of a solid culture of Sarcina lutea. The sarcinae were for the most part eliminated from the nasal membrane within 10 minutes in 2 persons, within 1 hour in a third, and within 24 hours in the other 2 persons. Swabs from the nasopharynx did not yield living sarcinae, except a few at 10 minutes or 1 hour in 3 of the 5 persons.

Bloomfield (1920 a) inoculated 4 persons on the nasal septum with a loopful of E.coli culture. Many living E.coli were found in the nose at 2 hours in all 4 persons, but none in any person at 24 hours. Living E.coli were found in the nasopharynx in small numbers at 10 minutes in 1 out of 4 persons, in large numbers at 2 hours in 2 out of 4 persons, and in small numbers at 24 hours in 1 out of 4 persons. Bloomfield also inoculated 3 persons on the nasal septum with a loopful of Staph.albus culture. Many living Staph.albus were found in the nose at 2 hours in all 3 persons, and a few at 24 hours in all 3 persons. Living Staph.albus were not found in the nasopharynx at 10 minutes; a few were found at 2 hours in all 3 persons, and a few at 24 hours in 1 out of 3 persons.

Bloomfield (1920 b) inoculated 5 persons on the nasal septum with a loopful of culture of one of three strains of H.influenzae. Many living H.influenzae were found in the nose at 10 minutes and at 2 hours in all/

all 5 persons, but none were found at 24 hours in 3 out of 5 persons, and none at 48 hours in any of the 5 persons. A few living H.influenzae were found in the pharynx of only 2 of the 5 persons at 2 hours, and of 1 of the 5 persons at 24 hours.

Bloomfield (1920 c) inoculated 6 persons on the nasal septum with a culture of Friedlander's bacillus. Many living bacilli were found in the nose of all 6 persons at 2 hours, but only a few in 1 out of the 6 persons at 24 hours. Living bacilli were not found in the pharynx at 10 minutes, a few or many were found in the pharynx of 4 out of 6 persons at 2 hours, and none in the pharynx of any person at 24 hours.

Bloomfield's observations on the nasal elimination of Sarcina lutea, E.coli, Staph.albus, H.influenzae and Friedlander's bacillus show that these bacteria have completely disappeared from the nose usually within 24 hours, some having been transported alive to the nasopharynx in the course of the first 2 hours and the majority apparently having been killed by a bactericidal action of the nasal secretion.

Arnold, Ostrom and Singer (1928) in 400 tests with 42 normal persons sprayed a suspension of about 20,000,000 E.coli or B.prodigiosus into the nostrils, and by making plate-cultures of swabs taken at intervals from the nasal membrane observed the rate of elimination of these bacilli. On average, 2000 colonies were obtained/

obtained from a swab taken just after spraying, 50 colonies at 5 minutes, 15 colonies at 10 minutes, 10 colonies at 15 minutes, 0 to 10 colonies at 30 minutes, and 0 colonies (rarely 1 or 2) at 60 minutes. This rapid elimination, 90 to 95% in 5 to 10 minutes, was due mainly to killing of the bacilli by the nasal secretion, since swabs taken from the posterior pharyngeal wall showed that few living bacilli were being discharged backwards out of the nasal cavity; only 25 to 40 colonies were obtained on a pharyngeal swab taken at 10 minutes, and few if any at 30 to 45 minutes.

Appleton (1944) has reviewed published studies of the mechanism of nasal elimination. The mucus film which constantly is being driven backwards over the ciliated epithelium, catches the bacteria and removes them mechanically to the pharynx; white blood cells escape on to the mucosal surface and dispose of the bacteria by phagocytosis; lysozyme, which is bactericidal towards certain species, is present in nasal secretion (Hilding, 1932 a,b; Linton, 1932; Daly, 1938).

De Waal (1941) made bacteriological observations of the nose and throat of a normal person exposed naturally to airborne infection in scarlet-fever wards. He found that the nose and throat very frequently became contaminated with haemolytic streptococci, presumably/

presumably by inhalation, but that this contamination was only temporary, the streptococci being eliminated rapidly by the defence mechanisms of the nose and throat. During a 36-day period while spending a large part of each day in scarlet-fever wards, de Waal made cultures from his nose and throat before and after each daily visit to the wards. On 32 out of the 36 days the nose culture was negative before entering the wards, and on 25 of these 32 days haemolytic streptococci were present in the nose on leaving the wards. On 23 of the 36 days the throat culture was negative before entering the wards, and on 5 of these 23 days haemolytic streptococci were present in the throat at the end of the ward visit. It was not determined whether the throat infection observed at the end of the ward visit was due to direct deposition of inhaled bacteria on to the throat or to backwards flushing to the throat of bacteria collected in the nose; at any rate, the nose was much more commonly infected than the throat, on 78% as against 22% of occasions, so without doubt the majority of the inhaled particles were deposited in the nose. Usually the contaminating haemolytic streptococci were eliminated within 18 hours. On 29 days haemolytic streptococci were present in the nose at the end of the ward visits; on only 2 of these 29 occasions was the same serological type of streptococcus found in the nose on the next morning (on/

(on 10 occasions the homologous type was found in the throat on the next morning, presumably having been flushed back from the nose). On 18 days haemolytic streptococci were present in the throat at the end of the ward visits; on only 3 of these 18 occasions was the same type of streptococcus found in the throat on the next morning. This study illustrates how a normal person may resist and dispose of repeated invasions by virulent haemolytic streptococci of various serological types.

Inhalation Infection Experiments with Animals.

Many investigators have found that infections with respiratory-tract pathogenic microorganisms can be induced in laboratory animals more readily and with smaller doses when administration is by inhalation than when it is by injection. Wells, Wells, Mudd, Lurie and Henle (1942) have reviewed investigations of experimental inhalation influenza, pneumonia and tuberculosis.

(16) Relative Importance of the Different Mechanisms of Transmission of Infection.

Opinion as to the relative importance of the different mechanisms of transmission of infection must be based firstly on an assessment of available information about the frequency of occurrence of pathogenic bacteria on the relevant vehicles, and secondly on epidemiological observations.

Food-borne and Milk-borne Infection.

Particularly in the case of food-borne and milk-borne outbreaks of infection does the epidemiological evidence indicate convincingly the mechanism whereby the infection is spread. Restriction of the infection to persons who had been served with a certain "dish" of food or supplied with milk from a particular source, is good proof that the food or milk in question was the vehicle of infection. In many investigations such a conclusion has been supported by isolation of the homologous type of pathogenic bacterium from the food or milk incriminated.

Well authenticated milk-borne outbreaks of scarlet fever and tonsillitis due to Strept.pyogenes include those described by Davies (1901), Newsholme (1902), Winslow (1912), Watson (1937), Henningsen and Ernst (1938) and Douglas, Smith, Sutherland and Watson (1941). Milk-borne outbreaks of diphtheria have been described by Dean and Todd (1902), Marshall (1907), Hercus, Shore, Barrett/

Barrett and North (1929) and others (see Chapin, 1912).

Among food-borne outbreaks, the tonsillitis-pharyngitis outbreak described by the Commission on Acute Respiratory Diseases (1945) is of especial interest. This outbreak of throat infections by the type-5 Strept. pyogenes was proved by the timing of cases and the dietary history to have been caused by the eating of creamed eggs at breakfast. It affected within ½ to 3 days, in the primary attack, 41.7% of a barracks community of 228 soldiers, giving a case to carrier ratio of 10 to 1. Of those soldiers who escaped the primary attack, 30.1% were infected after 3 days, presumably by contact or by air transmission from the primary cases; among these secondarily infected soldiers the case to carrier ratio was 1 to 1. The high case to carrier ratio of the primary attack was taken as indicating that all the infected persons had ingested an overwhelmingly large dose of the streptococci; the low case to carrier ratio of the secondary attack was taken as indicating that the airborne and contact mechanisms responsible for the secondary infections transmitted only small doses of the streptococci.

Food-borne and milk-borne epidemics of the respiratory infections do not, of course, account for more than a small proportion of the total incidence. The majority of cases must be due to other mechanisms of transmission.

Airborne Infection.

Observations of the bacterial content of the air have proved beyond doubt that small numbers of pathogenic bacteria usually are present in the air of rooms and other enclosed places which are occupied or visited by infected persons. If infection can be initiated by a small inoculum of pathogenic bacteria, such as a cluster of 1 to 100 on a single airborne particle, then the extent of commonly occurring air-contamination is sufficient to account in full for all endemic and epidemic spread of the respiratory infections. Sometimes it is argued that the infecting dose which is likely to be received by the airborne route will be too small to initiate infection, and that infection will result only from receipt of a large dose which supposedly can be provided more readily by direct contact or some other mechanism. It is very probable that some contact mechanisms are capable of transmitting especially large numbers of bacteria, for instance the "sharing", without intervening washing, of spoons, other eating utensils, apples and sweatmeats. However, the circumstances of such massive dosage are much too rare to account for the very high incidence of the respiratory infections and the great rapidity of their epidemic spread. Some forms of direct and indirect contact do occur very commonly, perhaps commonly enough to account for the rapid spread of respiratory infections/

infections; these include hand-shaking and the touching of commonly-handled objects; however, there are not any grounds for believing that these common forms of contact are likely to transmit larger numbers of bacteria than are transmitted through the air. Wells and Wells (1936) have argued that the high incidence and rapid spread of the respiratory infections are explicable only if infection is airborne.

Pertinent to the question of the size of an adequate infecting dose is the observation by Hamburger, Puck, Hamburger and Johnson (1944) that a person who visited certain hospital wards became infected under circumstances strongly suggestive of airborne infection by inhalation of a total of about 1300 haemolytic streptococci.

Making observations in measles and common respiratory disease wards, Hamburger, Puck, Hamburger and Johnson (1944) found that in each ward the incidence of cross-infections with each different serological type of haemolytic streptococcus was proportionate to the numbers of streptococci of that type found in the ward air. This suggests strongly that the cross-infections were caused by the airborne streptococci.

The most conclusive evidence regarding the importance of airborne infection is that obtained in studies of the influence on cross-infection rates of measures/

measures designed to prevent transmission through the air, such as air disinfection and air-space isolation in single-bed wards.

The importance of airborne infection is suggested by the failure of aseptic bed-isolation nursing to prevent cross-infections in multi-bed wards, as compared with the successful prevention of cross-infections by air-space isolation in single-bed wards. In early trials a certain measure of success was found to attend aseptic nursing in multi-bed wards; cross-infections were reduced in number, although not eliminated completely. The early trials were inadequate in that account was taken only of the cross-infections which were clinically manifest, and not of the much more frequent subclinical or "carrier" cross-infections. Recent studies have shown beyond doubt that aseptic nursing does not reduce greatly the incidence of cross-infections among patients occupying in common a multi-bed ward.

The early studies have been reviewed by Chapin (1912). Grancher (1900) nursed measles patients in common wards with other patients, paying strict attention to medical asepsis. During 10 years, measles was introduced into the wards 139 times and these cases gave rise to 115 cross-infections, less than a third as many as before the introduction of aseptic nursing.

Rundle/

Rundle and Burton (1912) during 2 years nursed patients with various infectious diseases along with other patients in a common multi-bed ward. They practiced aseptic bed-isolation nursing, but did not make any attempt at air-space isolation. In all, 668 persons passed through the ward, of whom 69 had scarlet fever, 40 diphtheria, 37 measles, 38 varicella, 9 whooping cough and 215 erysipelas. From all these there were only 2 clinically-manifest cross-infections: one of diphtheria and the other of scarlet fever.

Allison and Brown (1937) found that 57 out of 100 scarlet-fever patients who were nursed together in large multi-bed wards by the aseptic bed-isolation method, on discharge had present in the throat or nose a Strept.pyogenes of different serological type from that present on admission. On the other hand, none of 16 scarlet-fever patients who were nursed in completely separated single-bed cubicles, became cross-infected during their stay in hospital. In a newly opened 23-bed ward, scarlet-fever patients were examined twice weekly by swabbing of the nose and throat. Of 47 patients examined during 13 weeks, 33 (i.e. 70%) became cross-infected with a fresh serological type of Strept.pyogenes. Of the 33 cross-infected patients, 18 showed clinical signs of infection, "complications" such as fever, coryza, tonsillitis, cervical adenitis, otorrhoea or rash; of the 14 patients who escaped cross-infection/

cross-infection, only 2 showed clinically-manifest complications.

Stalker, Whatley and Wright (1942) similarly found that the bed-isolation nursing technique failed to prevent cross-infections with Strept.pyogenes occurring among scarlet-fever patients in multi-bed wards. Weekly throat swabs were taken from all patients and attendants, and the appearance of a fresh serological type of Strept.pyogenes was counted as a cross-infection. During a 4-month period the cross-infection rate was 20.3% among 74 patients in two 12-bed wards with nursing by ordinary methods, and 22.6% among 62 patients in two similar 12-bed wards with nursing by the most rigorous aseptic bed-isolation system. This aseptic nursing system must have eliminated completely the spread of infection by direct or indirect contact, or by fomites; yet it did not reduce the frequency of cross-infection. This suggests that under normal conditions infection was not commonly spread by contact or fomites. Another mechanism, almost certainly transmission through the air, must have been responsible for the majority of the cross-infections. The finding of numerous Strept.pyogenes in the floor dust suggested that dustborne air infection must have been common.

Wright, Shone and Tucker (1941) made similar observations in diphtheria wards. Cross-infections were observed by noting the appearance of a fresh type

of/

of the diphtheria bacillus (gravis, intermedius or mitis) in the weekly nose and throat cultures taken from each patient. During an 8-month period the cross-infection rate was 6.7% and 7.4% among 299 patients who were nursed by ordinary methods in two 26-bed wards, and 2.7% and 3.6% among 284 patients who were nursed by the aseptic bed-isolation system in two 20-bed wards. Aseptic nursing reduced the frequency of cross-infections, but did not eliminate them. On the other hand, cross-infections were eliminated by air-space isolation; there were not any cross-infections during a 10-month period among 82 patients nursed in single-bed cubicles which were completely separated by glass partitions. This cross-infection rate of 0% where airborne infection was prevented, contrasted markedly with the cross-infection rate of 13% observed among 70 patients nursed during a 6-month period in an ordinary open 13-bed ward. It may be concluded that air transmission was the main cause of the cross-infections.

A special application of the air-space isolation method was made by Chapple (1942) in nursing premature infants in separate incubators which were plenum-ventilated with outdoor air. Among 10 infants nursed with this protection against airborne infection the mortality was 0%, while among 10 infants nursed at the same time in an open ward with full aseptic precautions the/

the mortality from respiratory infections was 40%. Subsequently, 500 premature infants were nursed in incubators and only one of these developed a respiratory infection.

Wells (1944) has suggested that the importance of airborne infection in the spread of a disease may be assessed from the pattern of seasonal variation in the incidence of the disease. If infection is airborne, the rate of spread will be proportional to the concentration of the causative microorganisms in the inhabited atmospheres, and, since this concentration must vary with ventilation and thus with outdoor temperature, the incidence of the disease will be high in the cold season and low in the hot season; moreover, the seasonal variation in the disease rate will be greatest in the localities having the greatest seasonal variation in outdoor temperature. From a study of the disease rates reported in different localities in the U.S.A., Wells concluded that measles and chicken-pox probably are mainly airborne, but that scarlet fever is less affected by the amount of ventilation and so probably is not mainly airborne.

The finding that disinfection of the air reduces greatly the spread of a disease, is strong evidence that the disease is spread mainly by airborne infection. In particular, air disinfection by ultraviolet radiation, involving as it does irradiation of the upper room-air only, can not interfere with means of spread/

spread other than airborne, such as by contact or by projectile secretion droplets.

Wells, Wells and Wilder (1942) made a trial of air disinfection by ultraviolet irradiation as a means of preventing the spread of infectious diseases among children in day-schools. In several schools during 1 to 4 years, the classrooms of the younger children were irradiated, while the older classes were left unirradiated as controls. The air disinfection was found to reduce greatly the spread of measles, mumps and chicken-pox, indicating that these infections were mainly airborne. The measles attack rates among the susceptible children (excluding home secondary cases) were 14.5%, 15.7% and 9.0% for the irradiated classes and 55.3% and 51.8% for the unirradiated classes. During the first year in one school, 7 introductions of mumps into the irradiated classes were followed by only 2 secondary cases, while 7 introductions into the unirradiated classes were followed by 12 cases; during the second year, the irradiated classes suffered only 1 case of mumps, while the unirradiated classes suffered an epidemic of 32 cases. During the third year, 21 introductions of chicken-pox to irradiated classes gave only 7 secondary cases, while the exposure of one class to a missed case of chicken-pox for a short time in an unirradiated atmosphere caused infection of 15 out of the 16 susceptibles present.

Wells (1943) reported the results obtained on continuation of this investigation for a further two years; irradiation remained successful in reducing the spread of measles, but failed entirely to check an epidemic of mumps.

Perkins, Bahlke and Silverman (1947) attempted to repeat the observations of Wells, Wells and Wilder. Three large rural day-schools were studied; one was irradiated throughout, one in some classes only and one not at all. During an extensive measles epidemic which affected the locality in the first year, the overall morbidity among the susceptible children was 78% in the wholly irradiated school, 84% in the partly irradiated school (86% in the irradiated classes and 82% in the unirradiated classes), and 69% in the wholly unirradiated school. This failure of air disinfection in classrooms to reduce the incidence of measles was attributed to the children becoming infected while travelling to school together in the non-disinfected atmospheres of buses. However, the air disinfection seemed to modify the chronological pattern of the measles epidemic; in the unirradiated school the epidemic was explosive, intense and brief, while in the wholly irradiated school it was moderated and prolonged; in the partly irradiated school the unirradiated classes experienced a shorter and more intensive epidemic than the irradiated classes. Since the

irradiation had this moderating influence on the spread of measles, it was concluded that measles was airborne to an important degree.

Barenberg, Greene, Greenspan and Greenberg (1942) found that ultraviolet irradiation reduced the incidence of respiratory infections among infants nursed in multi-bed wards. The number of infections per child per month in three successive winters was 0.42, 0.59 and 0.62 in an irradiated ward, and 0.77, 0.78 and 0.85 in an unirradiated ward. In a chicken-pox epidemic none of the infants in the irradiated ward were affected, but 18 out of 19 infants in the unirradiated ward contracted the disease. During the first two winters the air disinfection was discontinuous, the ultraviolet lamps being operated for alternate ½-hour periods in the daytime only; it is difficult to understand how air disinfection could have been at all effective under such conditions.

Brooks, Wilson and Blackfan (1942) studied the influence of ultraviolet irradiation on the incidence of respiratory cross-infections in the multi-bed wards of an infants' hospital. During the first winter, when the ultraviolet lamps were operated in the daytime only, the irradiation appeared to reduce the frequency of cross-infections; the incidence of clinically-manifest cross-infections was 2.0% in two irradiated wards and 5.7% in two unirradiated wards. During the second winter, when the lamps were operated continuously/

continuously day and night, the irradiation failed to reduce the incidence of respiratory cross-infections; the incidence of clinically-manifest cross-infections was 4.5% in the irradiated wards and 4.4% in the unirradiated wards, while the incidence of culturally proved cross-infection with pathogenic bacteria was 19.4% in the irradiated wards and 22.0% in the unirradiated wards.

Wheeler and Jones (1942) reported that air disinfection by ultraviolet irradiation in certain wards of a rheumatic-fever hospital during one winter reduced the incidence of common colds (12% as compared to 25% in the unirradiated wards), but did not reduce significantly the incidence of sore throats (28% v.35%) or of rheumatic fever recurrences (16% v.13%).

Harris and Stokes (1945) reported the results of a 3-year trial of air-disinfection by glycol vapor in a children's convalescent home where most of the patients were confined to bed in 16-bed wards. Propylene glycol was vaporised during the first two winters and triethylene glycol during the third; in successive 3-week periods two similar groups of wards alternately were glycolised and left as controls. In the three winters the numbers of clinically-manifest respiratory infections were only 2, 5 and 6 in the glycolised wards as compared with 16, 100 and 16 in the control wards; morbidity had been reduced by 90% as a result/

result of the air disinfection. In the second winter the glycolised wards had 3 common colds and 2 other infections, while the control wards had 79 common colds and 21 other infections (i.e. pharyngitis, tracheo-bronchitis and otitis media).

Loosli, Smith, Gauld, Robertson and Puck (1947) made a 6-month trial of air disinfection by triethylene glycol vapor in a multi-bed hospital ward for infants. The rates per 1000 hospital-days of specific bacterial infections among infants not receiving chemotherapy were 7.5 for the glycolised ward and 12.4 for the control ward; this difference was not greater than might have been due to chance variation. Similarly, the rate of non-specific clinically-manifest cross-infections was only slightly lower in the glycolised than in the control ward.

These various trials of air disinfection as a means of preventing respiratory infections have not given concordant results. Some of the investigations were not sufficiently extensive in scope to preclude the possibility of gross error from chance-variation. Apart from this cause for discrepancy, the varying degrees of success and failure may well have been due to differences in the efficiency of the air-disinfection techniques employed and to differences in the amount of opportunity for transmission of infection through other atmospheres which were not disinfected. If these were the main reasons for the discrepancies, it seems

justifiable to accept the successful trials, those in which the morbidity rate was greatly reduced by the air disinfection, as evidence that the respiratory infections are mainly airborne.

Dustborne Air Infection.

If the practice of dust-suppression by the oiling of floors and bedclothes reduces the incidence of respiratory infections, it is strong evidence that the respiratory infections are spread by air carriage of infected dust-particles.

Anderson, Buchanan and MacPartland (1944) compared the incidence of respiratory infections (i.e. upper respiratory catarrh manifest by obvious local signs, or influenza with pyrexia) in two similar army units of 1300 to 1700 men. During a 17-week period, the average respiratory infection rate was 7 per 1000 men in the unit where the floors were oiled as compared with 38 per 1000 in the control unit.

Wright, Cruickshank and Gunn (1944) during 9 weeks in the spring compared the incidence of Strept.pyogenes cross-infections in two identical multi-bed measles wards. In one of the wards, the floor, blankets, sheets, counterpanes, pillow-cases, patients' garments, towels, gowns and curtains were treated with oil. Sulphonamides were administered prophylactically to all patients in both wards. Weekly swabs from the nose and throat revealed that cross-infection with a type-6 Strept.pyogenes/

Strept.pyogenes which was sulphonamide-resistant, occurred in 18.6% of patients in the oiled ward as compared with 73.3% of patients in the unoiled ward. The otitis-media complication rate, due to infection with the type-6 streptococcus, was 2.8% in the oiled ward and 14.3% in the unoiled ward.

Different results were obtained in a similar investigation made by Begg, Smellie and Wright (1947) during 19 weeks in the spring in the measles wards of another hospital. The Strept.pyogenes cross-infection rate among 190 measles patients nursed in the oiled ward was 20.5%, and among 186 patients nursed in the unoiled ward was 12.4%. The clinically-manifest complications due to streptococcal cross-infection were too few for valid comparison, there being only one case of otorrhoea in the two wards (0.27%). The oiling was fully effective in reducing the Strept.pyogenes content of the air (by 95% during bedmaking and by 96% during sweeping, as compared with the unoiled ward), so that the cross-infections seemingly must have occurred by some other route than through the air.

The Commissions on Acute Respiratory Diseases and on Airborne Infections (Dingle, Robertson et al., 1946) studied the effect of oiling floors and bedding on the incidence of respiratory diseases in a large army unit during one winter. The oiling effectively reduced the bacterial contamination of the air of the barracks, by 75% to 90%/

75% to 90% as compared with unoiled control barracks. During a period of low endemic occurrence of respiratory disease, the oiling appeared to reduce the incidence of hospitalised illness. During the epidemic occurrence of acute undifferentiated respiratory disease, oiling had little or no effect.

Shechmeister and Greenspan (1947) during 13 months studied the effect of oiling floors and bedding in the multi-bed dormitories of about 2400 naval personnel in a training barracks. Although reducing the bacterial content of the air by 33% to 63%, oiling only slightly reduced the incidence of respiratory disease during periods of low incidence, and did not have any effect on the incidence of minor respiratory complaints.

These various trials of dust-suppression as a means of preventing respiratory infections have not given concordant results and it is not possible to base on them any reliable conclusions as to the importance of dustborne infection. However, direct bacteriological examinations of the air of occupied rooms have shown clearly that dust-raising is the most important means whereby air becomes contaminated with pathogenic bacteria. Assuming that the respiratory-tract infections are mainly airborne, the primary importance of dust infection is apparent.

Infection by Contact, Fomites and Projectile Droplets.

Epidemiological observations have been reported from/

from time to time which supposedly prove that in particular instances an infection has been transmitted by contact or by fomites or by projectile secretion-droplets. These "demonstrations" have been of little value. Most were made in the absence of modern knowledge about the other likely mechanisms of spread and in ignorance of the possibility that healthy carriers may act as a source of infection. When it was believed that each case of a disease must have been caused by transmission from a previous known case, the circumstances of relationship between the two cases seemed convincingly to indicate the mode of transmission. However, if a disease can be contracted from an unknown healthy carrier, the mode of transmission can not be discovered and proved in this way.

As examples of supposedly proved transmission by fomites, two of the many cases quoted by Chapin (1912) may be given in illustration: a doctor was supposed to have caught scarlet fever from a coat which he wore while attending a scarlet-fever case some 18 months previously; several children were supposed to have contracted diphtheria from a trumpet which had been used by a diphtheria patient 4 years previously!

Evidence that projectile secretion droplets are an important means of infection, was thought to be provided by observations of the influence of bed-spacing-distance on the frequency of infections in multi-bed/

multi-bed dormitories. Glover (1918 b), investigating army barracks, found that spacing-out of the beds to a distance of 2½ feet (together with improvement of ventilation) reduced the meningococcus carrier-rate from an average of 25% to an average of 4%. This was taken as indicating that the "range" of droplet-spray is not more than 2½ feet. However, since the spacing-out of beds may influence the infection rate in many ways other than in relation to the droplet-spray range, such "demonstrations" of the importance of droplet infection are invalid.

(17) Conclusions.

It is thought that the most important mode of spread of the respiratory-tract infective diseases is by dustborne infection through the air. As a result of blowing and picking the nose, wiping and fingering the mouth, sneezing, coughing, speaking and spitting, large numbers of pathogenic bacteria are distributed from the nose and throat of infected persons on to their hands, handkerchiefs, clothing and bed-linen, and on to the floors and furniture of their rooms. These bacteria, which are capable of surviving for a few hours to a few months the room conditions of light and drying, are liberated into the air by any movement or disturbance: from the skin and clothing by ordinary body movements, from the handkerchief by the movements of taking it from the pocket and shaking it open, from the bed-linen by bed-making and by movements of the person in bed, from furniture by dusting, and from floors by sweeping and by the tramping of feet. The infected dust-particles remain suspended in the air for a few minutes to a few hours, are transported by air currents throughout the room of their origin and perhaps also to other rooms in the same building, and are inhaled by persons occupying these rooms, being deposited mostly in the nose and nasopharynx, and only rarely in the lungs.

Probably of less importance as a means of infection is/

is transmission of the pathogenic bacteria by contact or by projectile secretion droplets, perhaps indirectly by fomites or by flies, to the hands, lips, food or eating utensils of the recipient, and thence into his mouth and throat.

A mode of spread which probably is rare, is direct airborne infection by the secretion spray expelled in speaking, coughing and sneezing, that is by droplet nuclei which remain airborne for a few minutes to a few hours and are inhaled.

# Part 1b

## THE TRANSMISSION OF RESPIRATORY-TRACT TUBERCULOSIS

(1) Incidence and Importance of Pulmonary Tuberculosis.

There is no doubt that pulmonary tuberculosis is one of the most important causes of sickness and death in temperate climates and urban communities. Cobbett (1917) gave the annual death rate per million in the year 1910 as being 587 in New Zealand, 700 in Australia, 972 in Belgium, 1015 in England and Wales, 1142 in Scotland, 1174 in Italy, 1189 in Netherlands, 1421 in Germany, 1716 in Ireland, 1788 in France, 2880 in Austria, 3437 in Serbia and 3480 in Hungary. During the hundred years preceding the 1939-45 war, there was a steady decline in the mortality rate from pulmonary tuberculosis; the annual death rate per million persons living in England and Wales was 2890 in the years 1851-55, 2327 in 1871-75, 1504 in 1891-95, 1005 in 1911-15, 620 in 1931-35, and 477 in 1938 (see Wilson and Miles, 1946). Even during the pre-war years when the mortality was at its minimum, it was still very great. The total number of deaths from respiratory-tract tuberculosis during the ten years, 1931-40, as reported by the Registrar-General (1944 a, b), was 243,734 in England and Wales, and 28,410 in Scotland, representing 4.9% and 4.3% of the total number of deaths from all causes in the same period; one death in twenty was due to pulmonary tuberculosis. Yet the full importance of the disease is not revealed by these figures for deaths at/

at all ages. Deaths of young adults represent a greater loss to the community than deaths of persons at other ages, particularly of old persons. In the especially valuable 15-30 years age-group, one death in three was due to respiratory-tract tuberculosis. About 75% of all deaths from respiratory tract tuberculosis occur between the ages of 15 and 50 years (see Topley and Wilson, 1936; p. 1012, table).

Apart from the high mortality, an important amount of sickness and incapacity is caused. For example, during the three years, 1934-37, out of a total of 64,997,542 days of incapacity from all causes in the insured population of Scotland (about 1,700,000), 2,619,383 days of incapacity, or 4%, were due to respiratory-tract tuberculosis (Reports, 1936, 1937, 1939).

Since infection frequently remains latent, these mortality and morbidity figures do not reveal the total incidence of tuberculous infection of the respiratory tract. From the investigations of von Pirquet (1907), Mantoux (1910) and Calmette, Grysez and Letulle (1911) by the tuberculin test, of Naegeli (1900) and Burkhardt (1906) by post-mortem examination, and of Hetherington, McPhedran, Landis and Opie (1929), and many others, by radiological examination, it has been concluded that in urban communities between 10% and 20% of persons at all ages/

ages are, at any one time, suffering from some form of latent but active tuberculous infection, and that the great majority, over 90%, of all persons become infected with tuberculosis before attaining adult life (Wilson and Miles, 1946). The majority of the tuberculous infections are situated in the respiratory tract. The incidence of latent pulmonary tuberculosis is given by Opie (1917, a, b; 1924 a, b; 1925) from observation of healed focal and apical tuberculous lesions of the lungs among persons in St. Louis who died of diseases other than tuberculosis; the incidence was 42.8% for persons of 2 to 5 years of age, 45.5% for 5-10 years, 55.5% for 10-18 years, 83.3% for 18-30 years, 91.3% for 30-50 years, and 93.3% for 50-70 years (see Wilson and Miles, 1946). At some time during his life, almost every member of an urban community contracts tuberculous infection of the lungs. A rather lower incidence of infection has been reported for a semi-rural population in recent years; Landé and Wolff (1941) in Maryland found that only 48.4% of 128 persons over 20 years of age coming to post-mortem examination showed pathological evidence of tuberculous infection.

(2) Relative Importance of Exposure and Resistance in Determining the Incidence of Pulmonary Tuberculosis.

The probable value of different preventive measures in reducing the incidence of pulmonary tuberculosis depends largely on the extent to which that incidence is determined by degree of exposure and the extent to which it is determined by degree of resistance. If the incidence of pulmonary tuberculosis in a given community is being limited by and varying with the degree of exposure, that is the frequency with which persons encounter tubercle bacilli (i.e. inhale the bacilli into the lungs), then the most effective preventive measures will be those directed towards reducing the degree of exposure by minimising the environmental distribution of tubercle bacilli. If, on the other hand, the incidence of pulmonary tuberculosis in the community is being limited mainly by the resistance to infection possessed by the individual persons, then the most effective preventive measures will be those directed towards increasing such resistance to infection, as for instance the betterment of nutrition.

With regard to incidence of disease, degree of exposure and degree of resistance, three relationships are possible. Firstly, tubercle bacilli might be so widely distributed and so frequently encountered by/

by all members of the community, that the incidence of pulmonary tuberculosis would depend entirely on the proportions of resistant and non-resistant persons. Secondly, all members of the community might have such a low degree of resistance that each would contract clinically-manifest pulmonary tuberculosis at his first encounter with the tubercle bacillus, and the incidence of the disease thus depend entirely on the extent to which tubercle bacilli were distributed from patients throughout the communal environments. Thirdly, there might be an intermediate relationship with the disease incidence varying both according to the degree of exposure and according to the degree of resistance; this relationship will occur if there is in the community a variation between susceptibility and resistance in different persons, as according to age and nutrition, and if, in addition, exposure is naturally limited to the extent that a proportion of the susceptible persons normally remain uninfected through failure to encounter the tubercle bacillus.

The available epidemiological evidence indicates that the incidence of pulmonary tuberculosis in fact does vary, according to this third relationship, both with the degree of exposure and with the degree of resistance.

That/

That the degree of resistance influences the incidence of pulmonary tuberculosis, is proved beyond doubt by the very common occurrence of latent tuberculosis in persons who remain healthy and never exhibit clinical symptoms of consumption; the tuberculin test findings of von Pirquet (1907), Mantoux (1910) and Calmette, Grysez and Letulle (1911) and the necropsy findings of Naegeli (1900) and Burkhardt (1906) show that over 90% of persons in urban communities encounter the tubercle bacillus within the first 20 years of life and, because of adequate resistance, develop merely a latent infection instead of manifest consumption.

Evidence indicating that the degree of exposure influences the incidence of pulmonary tuberculosis, is abundant and convincing in the case of children, but less so in the case of adults.

Many investigators have shown that the incidence of latent, clinically-manifest and fatal tuberculosis is much greater among children living in families with a consumptive member who discharges tubercle bacilli in his sputum, than among children living in families with a consumptive member who does not discharge tubercle bacilli (e.g. Cox, 1929; Brailey, 1940). Opie, McPhedran and Putnam (1935) found that among children who were first exposed between 0 and 9 years of age to contact with a consumptive patient and/

and who lived for 12 to 14 years after first exposure, pulmonary tuberculosis was contracted by 9.9% of those exposed to a sputum-positive patient and by only 2.0% of those exposed to a sputum-negative patient; that among children who were first exposed between 10 and 14 years of age to contact with a patient and who lived for 10 to 14 years after first exposure, pulmonary tuberculosis was contracted by 20% of those exposed to a sputum-positive patient and by 0% of those exposed to a sputum-negative patient; and that among persons first exposed after 15 years of age, pulmonary tuberculosis was contracted by 9.7% of those exposed to a sputum-positive patient and by 6.9% of those exposed to a sputum-negative patient. These figures suggest that children acquire pulmonary tuberculosis mainly in the home.

With regard to adults, some investigators report an exceptionally high frequency of pulmonary tuberculosis among those, such as nurses and medical students, who are exposed unusually much to consumptive patients (e.g. Heimbeck, 1927, and many others quoted by Wilson and Miles, 1946), while other investigators report a normal incidence of pulmonary tuberculosis in such frequently-exposed adults (see Rogers, 1920).

The virtually universal occurrence of either latent or manifest tuberculosis in childhood suggests that/

that pulmonary tuberculosis in the adult may have an endogenous origin, occurring in a period of lowered resistance by reactivation of a healed childhood lung lesion or by haematogenous spread from a childhood lesion elsewhere in the body. If adult consumption arises by endogenous infection, the frequency of exposure to exogenous infection can have little influence on the incidence of the disease. However, it has been shown that pulmonary tuberculosis of the adult is not merely the reactivation of a childhood lung lesion; Opie (1917 a, b; 1924 a, b; 1925) found that the apical lesions of the adult disease did not have the same anatomical distribution within the lungs as the focal lesions of the childhood disease, the apical and focal lesions occurring in opposite lungs in half the cases examined. The possibility remains that the adult lung infection has an endogenous origin by blood spread from a childhood lesion elsewhere, but the epidemiological evidence regarding consumption in adults who have been subject to tuberculous infection in childhood, indicates that exogenous infection plays an important part. Observing 932 adults over 14 years of age who had had a positive tuberculin reaction in childhood, Israel and DeLien (1942) found that in white persons the mean annual morbidity rate was 2% of those exposed to household infection after 14 years of age, but only 0.1% of those not exposed to household infection.

It is concluded that the influence of the degree of exposure on the incidence of pulmonary tuberculosis, certainly is very great in the case of children, and probably is considerable in the case of adults.

(3) Types of Tuberculosis.

According to their aetiology and pathogenesis, two main types of primary tuberculosis are distinguished, respiratory and alimentary; secondary tuberculosis of other parts of the body occurs by spread from the primary lesion in the respiratory tract or the alimentary tract.

The two main aetiological types of tuberculosis are: (i) primary pulmonary tuberculosis (of lungs and bronchial glands) which is due to the human-type tubercle bacillus and acquired usually by inhalation of airborne infected particles originating from humans with open pulmonary tuberculosis; and (ii) primary alimentary tract tuberculosis (of tonsils and cervical glands, or intestine and mesenteric glands) which is due to the bovine-type tubercle bacillus and is acquired by ingestion of milk from a tuberculous cow.

Rare aetiological types of tuberculosis include: (iii) primary pulmonary tuberculosis due to bovine-type bacilli probably inhaled from air contaminated by tuberculous cows; (iv) primary alimentary tract tuberculosis due to ingestion of human-type bacilli introduced into the mouth on hands, utensils or food contaminated by a patient, or perhaps inhaled, caught in the upper respiratory tract and swallowed; (v) primary infection through the skin, nose or conjunctiva; and (vi) congenital infection.

Some/

Some cases occur of secondary pulmonary tuberculosis due to a bovine-type bacillus spreading from a primary alimentary-tract lesion, and many cases occur of secondary alimentary-tract tuberculosis due to a human-type bacillus swallowed in sputum from a primary lung lesion.

The evidence which establishes the validity of the two main aetiological types of tuberculosis, and also the evidence concerning the other, less common modes of infection, will be reviewed later when considering the manner of acquisition of infection.

The present review is concerned only with the respiratory type of tuberculosis and with the mechanisms whereby human-type tubercle bacilli are transmitted from a tuberculous person to the respiratory tract of a healthy person.

(4) Historical Summary of Investigations and Theories on the Mechanism of Transmission.

Following his discovery of the tubercle bacillus, Koch (1884) demonstrated that laboratory animals readily contract pulmonary tuberculosis through inhalation of air which has been contaminated by mechanical atomisation of a tubercle-bacillus culture suspension. On the basis of his observations, Koch defined two main possible mechanisms of transmission, namely by airborne cough-droplets and by airborne dried sputum dust-particles; he attributed greater importance to the "dustborne infection" mechanism. Koch wrote: "there can likewise be no doubt as to the manner in which the tuberculous virus is carried from phthisical to healthy subjects. By the force of the patient's cough particles of tenacious sputum are dislodged, discharged into the air, and so scattered to some extent. Now numerous experiments have shown that the inhalation of scattered particles of phthisical sputum causes tuberculosis with absolute certainty, not only in animals easily susceptible to the disease, but in those also which have much more power of resisting it. It is not to be supposed that man would be an exception to this rule, but, on the contrary, we may surmise that any healthy person brought into immediate contact with a phthisical patient, and inhaling the fragments of fresh sputum discharged into the air, may thereby be infected. But/

But probably infection will not often take place in this way, because the particles of sputum are not small enough to remain suspended in the air for any length of time. Dried sputum, on the contrary, is much more likely to cause infection, as, owing to the negligence with which the expectoration of phthisical patients is treated, it must evidently enter the atmosphere in considerable quantity. The sputum is not only ejected directly on the floor, there to dry up, to be pulverised and to rise again in the form of dust, but a good deal of it dries on bed-linen, articles of clothing and especially pocket handkerchiefs - which even the cleanliest patients can not help soiling with the dangerous infective material when wiping the mouth after expectoration - and also is subsequently scattered as dust."

The theory that infected dust was the most important means of infection was elaborated by Cornet (1889), who made numerous observations of the presence of tubercle bacilli in the dust of the rooms of phthisical patients. In opposition to this, Flügge (1897 a, b; 1899; 1901) developed the theory that infection usually was spread by cough-spray; he showed that mechanical atomisation of bacterial suspensions charged the air with "floating" infected particles, and his pupils demonstrated that infected particles were discharged from the mouth by coughing.

Thus/

Thus initiated, the "dust versus droplets" controversy has been continued until the present day. Quantitative observations have been made of the extent of the dissemination of tubercle bacilli in dust and in cough-spray, and of the relative ease with which air can be contaminated and laboratory animals infected by each of these agents; noteworthy are the investigations on dust infection of Tappeiner (1880), Cornet (1889, 1899), Schnirer (1891), Praussnitz (1891), Bissel (1895, 1899), Sticher (1899), Beninde (1899), Heymann (1901), Coats (1901), Hill (1902), Gotschlich (1903), Wagner (1903), Köhlisch (1908), Friberger (1908-09), Chaussé (1913 c, d; 1914 c), Sweany and McLane (1919), Brown, Petroff and Pesquera (1919-20), Rogers (1920), Lange and Nowoselsky (1925), Lange (1926), Augustine (1929), Horwood (1931), Sim and Flinn (1939), Smith (1942), and Smith, Urabec and Mason (1946), and those on droplet infection of Koch (1884), Flügge (1897 a, b; 1899; 1901), Laschtschenko (1899), Heymann (1899, 1901), Goldie (1899), Koelzer (1903), Bernheim (1905), Ziesché (1907), Findel (1907), Reichenbach (1908), Chaussé (1914 a, b; 1916), Kenwood and Dove (1915), Rogers (1920), Hippke (1921), Lange and Nowoselsky (1925), Sim and Flinn (1939), Wells and Lurie (1941), Wells, Ratcliffe and Crumb (1948) and Lurie and Abramson (1948). That the tubercle bacillus can survive drying and daylight sufficiently long to permit/

permit frequent infections by dust, was shown by the findings of Migneco (1895), Heymann (1901), Hill (1902), Twitchell (1905), Kirstein (1905), Cadéac (1905, 1908), Weinzirl (1907), Rickards, Slack and Arms (1909), Chaussé (1912; 1913 c, d, e), Kenwood and Dove (1915), Caldwell (1925), Lange (1926), and Smith (1942, a, b). Any decision as to the relative importance of dust and droplet-spray must be based on an assessment of the results of these investigations. Attention must be drawn to the outstanding importance of Chaussé's work, which ranks as the foremost contribution to this subject on account of its comprehensive scope, the careful planning of experiments and the judicial assessment of results; in spite of this, the work of Chaussé has been almost completely ignored in the English-language medical literature.

While it was found by early investigators that small, artificially-produced, bacteria-carrying droplets may remain airborne for up to several hours and be transported by air currents to considerable distances within a building (Flügge 1897 a, b; 1899, 1901; Koeniger, 1900; Hutchinson, 1901), it was concluded from direct observations of the cough-spray of tuberculous persons that the naturally-produced infected droplets are mainly large and fall out of the air within a few seconds after expulsion and within the range of a few feet (Flügge 1899, 1901; Laschtschenko,/

Laschtschenko, 1899; Heymann, 1899, 1901). The investigations of Chaussé (1913 a, b) revealed that there is a clear-cut distinction according to size between, on the one hand, the large projectile droplets, of 200 to 2000 microns in diameter, which do not remain airborne for more than a few seconds and can not be inhaled deeply into the respiratory tract, and, on the other hand, the small droplets which at once become further reduced in size by desiccation and so form dry particles of 2 to 15 microns diameter (the "droplet nuclei" of Wells, 1934) capable, because of their small mass, of remaining suspended in the air for a long time, of being transported to considerable distances and of being inhaled deeply into the lungs. Lange and Keschischian (1925) confirmed this distinction. Wells (1934) made clear the exact size-relationships and the other physical factors on which the distinction depends, and Wells and Wells (1936) drew attention to the great possibilities for transmission of respiratory infections by the "droplet nuclei."

While the majority of investigators, including Koch, Cornet and Flügge, have taken it for granted that pulmonary tuberculosis is contracted by direct inhalation to the lungs of airborne infected particles, some investigators, notably Woodhead (1894), von Behring (1903) and Calmette and Guérin (1905; 1906 a, b), have argued, from pathological observations/

observations and the results of experiments with animals, that pulmonary tuberculosis is caused by ingestion of infected material with subsequent passage of the tubercle bacilli through the mucosa of the alimentary canal, via the lymphatics and blood stream, to the lungs. If infection can be acquired by ingestion, then it is possible that infection may be transmitted by contact, by eating utensils or by the irrespirable large projectile droplets of cough-spray, instead of necessarily by respirable airborne dust-particles or droplet-nuclei; tubercle bacilli spread by contact or by projectile droplets must necessarily enter the body through the mouth, as on contaminated fingers, utensils or food, and can hardly pass directly to the alveoli of the lungs. The relative importance of, on the one hand, inhalation infection, and thus airborne infection, and, on the other hand, ingestion infection, and thus contact infection, can be assessed from evidence of five kinds: firstly, from observations of the frequency of occurrence of tubercle bacilli in the air of dwellings occupied by tuberculous persons (Le Noir and Camus, 1908 a, b, 1909; Chapin, 1912; Chaussé, 1914 c; Cumming, 1920; Augustine, 1929; Pressman, 1937; Eisenberg, 1937; Sim and Flinn, 1939; Bogen and Dunn, 1941; Smith, Urabec and Mason, 1946), and of the frequency of their occurrence on vehicles of contact infection such as hands, eating utensils and fomites (Baldwin,/

(Baldwin, 1898; Dieudonné, 1901; Mitulescu, 1903; Huhs, 1906; Ostermann, 1908; Klein, 1908; Price, 1908; Graziani - see Rosenau, 1908; Kenwood and Dove, 1915; Cumming, 1920; Rogers, 1920; Brown, 1921-22; Taylor, 1921-22; Augustine, 1929); secondly, from animal experiments designed to show whether and how readily it is physically possible for bacteria to pass directly in the inspired air to the lung alveoli (Nenninger, 1901; Calmette and Guérin, 1905; Hartl and Hermann, 1905; Bartel and Neumann, 1906; Kovacs, 1906; Köhlisch, 1908; Cobbett, 1910; Chaussé, 1913 b; Lange and Keschischian, 1925; Wells and Lurie, 1941; Hatch, 1942; Lurie and Abramson, 1948), from the tonsils to the lungs (Grober, 1905; Wood, 1906; Winternitz, Smith and Robinson, 1920), and from the stomach or intestine to the lungs (van Steenberghe and Grysez, 1905; Whitla, 1908; Schlossman and Engel, 1906; Ravenel and Reichel, 1908; Griffith, 1907, 1911; Cobbett, 1910); thirdly, from animal experiments designed to show the relative ease of infection by inhalation and by ingestion (Gebhardt, 1890; Preyss, 1891; Kossel, Weber and Heuss, 1905; Findel, 1907; Reichenbach, 1908; Weber and Titze, 1910; Lange, 1924; Lange and Nowoselsky, 1925; Wells, Ratcliffe and Crumb, 1948) and to show whether tuberculous lesions, particularly primary lesions, may be produced in the lungs as a result of ingestion of tubercle bacilli (Calmette and Guérin,/

Guérin, 1905, 1906 a, b; Griffith, 1907; Lange, 1924); fourthly, from observations of the anatomical distribution of the primary-infection complex in human tuberculosis necropsies (Ghon, 1916; Opie, 1917 a, b, 1924 a, b, 1925; Griffith, 1925); and fifthly, from observations of the relative frequency of occurrence of human-type and bovine-type tubercle bacilli in pulmonary and other tuberculous lesions (Griffith, 1914; Cobbett, 1917; Lange, 1932; Blacklock, 1932; Griffith and Munro, 1943; Cutbill and Lynn, 1944).

(5) Definition of Possible Mechanisms of Infection.

The mechanism whereby tuberculous infection of the lung is acquired may be considered in two stages:-

A. the transmission of the tubercle bacilli and their entry into the body of the recipient, and

B. the passage of the tubercle bacilli to the lungs of the recipient.

A. WAYS OF TRANSMISSION AND ENTRY INTO THE BODY.

1) Inhalation of airborne infected particles, either
   a. dust particles formed by desiccation of patient's sputum or saliva, as smears or projectile droplets, on clothes, handkerchiefs, bedding, floors and furniture; or
   b. droplet nuclei produced by patient's cough, sneeze or speech spray.

2) Ingestion of tubercle bacilli into the mouth,
   a. in kissing patient, or
   b. on hands, eating utensils or food contaminated
      i. by contact with patient or infected fomites,
      ii. by flies feeding on patient's sputum, or
      iii. by projectile droplets of patient's cough sneeze or speech spray.

B. WAYS OF PASSAGE TO THE LUNGS./

B. WAYS OF PASSAGE TO THE LUNGS.

1) By direct inhalation to the lung alveoli of small airborne infected particles in the inspired air.

2) By preliminary collection in mouth, throat or nose of tubercle bacilli acquired either

i. by inhalation of large airborne particles,

or

ii. by ingestion of bacilli into mouth,

and with subsequent passage to the lungs:-

2a) By aspiration via trachea to lungs, of autogenous droplets or small masses of the contaminated secretion of the recipient's own mouth, throat or nose;

2b) By invasion of tonsil or adenoid with passage via cervical and mediastinal lymphatics, or via the blood stream, to the lungs; or

2c) By swallowing to intestines with passage through mucosa and via mesenteric and mediastinal lymphatics, or via the blood stream, to the lungs.

(6) Sources of Human-Type Tubercle Bacilli.

The ultimate source of human-type tubercle bacilli, the site of their growth and multiplication, is the infective lesion in a tuberculous person. There are not any true "carriers" of tubercle bacilli, though persons with latent active tuberculosis may sometimes disseminate the bacilli. Almost certainly, contagion originates mainly from clinically-manifest cases of the disease.

Bacilli from lung lesions may be discharged in sputum, saliva and faeces, bacilli from alimentary-tract lesions may be discharged in faeces, bacilli from kidney lesions may be discharged in urine, bacilli from tuberculous lymph-glands, abscesses and other lesions may be discharged through sinuses in pus, and, possibly, bacilli from infrequent tuberculous lesions in the nasopharynx and nasal cavity may be discharged in nasal exudate. Thus, sputum, saliva, nasal exudate, pus, faeces and urine must be considered as possible vehicles of human-type tubercle bacilli. Those who have studied the mechanism of transmission of tuberculosis have confined their attention almost entirely to sputum and saliva, and have disregarded nasal exudate, pus, faeces and urine; this preoccupation with sputum and saliva is probably justified since these fluids appear of much greater importance than the other possible vehicles/

vehicles of infection. Faeces and urine are usually disposed of expeditiously by use of sanitary conveniences in a manner which precludes free distribution of their bacillary content within dwellings. Discharge of infected pus and infected nasal exudate is much less common than discharge of infected sputum and infected saliva.

Sputum containing tubercle bacilli is discharged by most persons suffering from clinically-manifest, active tuberculosis of the lungs, but usually not by persons with latent lesions or healed lesions of the lungs. Vogt, Zappasodi and Long (1940) found tubercle bacilli in 617 (i.e. 71%) of 880 specimens of sputum from 296 consumptive patients.

Chaussé (1914 a) estimated the number of tubercle bacilli in sputum from counts made by microscopical examination of Ziehl-stained smears of a measured volume of the sputum spread over a measured area of slide; he found that the sputum of a number of patients with advanced pulmonary tuberculosis contained from 5,000,000 to 120,000,000 tubercle bacilli per ml., the sputum of most patients containing between 5,000,000 and 30,000,000 per ml.. Examining 12 patients whose sputa were rich in tubercle bacilli, Chaussé (1914 b) found between 15,000,000 and 125,000,000 per ml., on average 63,000,000 per ml. Examining the sputa of 24 patients who happened to occupy/

occupy a certain common ward, Chaussé (1914 c) found that the content of tubercle bacilli varied in the different patients from 1,000,000 to 160,000,000 per ml., being on average 60,000,000 per ml. The sputa of a further 21 patients, chosen as being highly infective, were found by Chaussé (1916) to contain from 80,000 to 106,000,000 tubercle bacilli per ml., on average 30,000,000 per ml. The patients studied by Chaussé were recorded as expectorating 100 to 400 ml. of sputum per day; the output of tubercle bacilli in these cases must often have been about 10,000,000,000 per day.

Recent investigators have estimated the bacillary output in sputum by microscopical counting of the bacilli in a stained smear prepared from a homogenised centrifuge-deposit of an alkali-treated or trypsin-digested 24-hour specimen of the sputum; such a method enables more accurate counting of the bacilli, especially when only a small number are present in the sputum. Jordan (1938) examined 35 three-day specimens of sputum and found that the three-day output varied from 100,000 to 400,000,000 tubercle bacilli, being often about 10,000,000. Bogen and Bennett (1939) examined 300 one-day specimens of sputum collected from inmates of a sanatorium; in 79 cases the 24-hour output was about 10,000,000 tubercle bacilli, in 51 cases about 1,000,000 bacilli, in 27 cases about 100,000 bacilli, in 13 cases about 10,000/

10,000 bacilli, and in 130 cases not any bacilli. Vogt, Zappasodi and Long (1940) examined 158 one-day specimens of sputum from 37 white patients with pulmonary tuberculosis; 45 specimens did not contain any tubercle bacilli, while 113 specimens contained tubercle bacilli in numbers such that the average 24-hour output was 129,593,000, the highest 24-hour being 1,680,000,000. These authors also examined 722 specimens from 259 negro patients; 218 specimens did not contain any tubercle bacilli, while 504 specimens contained tubercle bacilli in such numbers that the average 24-hour output was 893,940,000, the highest 24-hour output being 20,499,918,000. Typical counts shown, were from 1,000,000 to 10,000,000 per ml. of sputum.

From these various reports it may be concluded that a majority of patients with manifest pulmonary tuberculosis disseminate tubercle bacilli in their sputum to the extent of 10,000 to 100,000,000 per ml., and of 100,000 to 10,000,000,000 per day.

Saliva does not commonly become infected from tuberculous lesions of the mouth since such lesions occur only rarely. Appleton (1944) has reviewed studies of the incidence of oral tuberculosis, lingual, labial and pharyngeal; in numerous published series, in all comprising about 67,000 tuberculosis cases and necropsies, tuberculosis of the oral cavity was found only/

only in 127 cases.

Tuberculosis of the tonsils is fairly common, but the lesions are usually of slight extent, being apparent only on microscopical examination, so that they may rarely contribute tubercle bacilli to the mouth secretions. Newhart, Cohen and van Winkle (1934) reported an incidence of tuberculosis of the tonsils in 2.03% of 30,676 tonsillectomies in a collected series. A high incidence of secondary tonsillar tuberculosis occurs in persons with open pulmonary tuberculosis; Vlasto (1931) quoted Strassmann as having found at post-mortem examination tuberculosis of the tonsils in 13 out of 21 patients with advanced pulmonary tuberculosis; Rosencrantz and Hurwitz (1941) by microscopical examination found the incidence of tuberculosis of the tonsils among 103 tuberculous children to be about 18%.

Quite apart from the existence of oral or tonsillar lesions, tubercle bacilli have been found frequently to be present in the mouth secretions of patients with open pulmonary tuberculosis; the surfaces of the mouth become infected by sputum during the course of expectoration and the tubercle bacilli may not be removed by the clearing-mechanisms of the mouth for more than an hour following the expectoration. It may be noted that in addition to the mechanical clearing mechanisms of the mouth, the saliva/

saliva has been found to exert an antibacterial action on the tubercle bacillus (Kanter and Appleton, 1940). Weissmayr (1898) examined microscopically 19 specimens of saliva from 6 patients with open pulmonary tuberculosis and found tubercle bacilli in 5 specimens from 4 of the patients, but only in very small numbers (1, 1, 1, 15 and 24 bacilli respectively). Laschtschenko (1899) examined microscopically secretion swabbed from the inside of the cheeks when no sputum was present; he found tubercle bacilli in this oral secretion in the case of 9 out of 20 patients with pulmonary tuberculosis. Park (1908) found tubercle bacilli in the saliva of 10 out of 15 cases, and quoted Möller as finding tubercle bacilli in the saliva of 3 out of 20 cases. Neild and Dunkley (1909) examined microscopically saliva scraped from the anterior part of the tongue of patients with pulmonary tuberculosis in whose sputum tubercle bacilli had been demonstrated; they found tubercle bacilli in the saliva of 26 out of 50 patients, including some who had not expectorated for an hour or so before examination. Chaussé (1914 a) noted that the saliva's content of tubercle bacilli is dependent on contamination with sputum and must vary from time to time according to the occurrence of expectoration. He examined microscopically samples of saliva collected at various times in relation to spitting (never just after rinsing the mouth) from 20 patients whose sputum contained numerous tubercle bacilli. He found/

found tubercle bacilli in the saliva of 7 patients (35%) but not in the saliva of the other 13 patients. The numbers of tubercle bacilli in these infected saliva samples were 120 to 25,000 times less than the numbers present in the sputum of the same patients. Chaussé concluded that the saliva of open pulmonary tuberculosis patients who expectorate, is always infective, but that this saliva is 100 to 100,000 times less infective than the sputum. Rogers (1920) by guinea-pig inoculation found virulent tubercle bacilli in the saliva of 4 out of 5 patients with open pulmonary tuberculosis. Gloyne (1922) found virulent tubercle bacilli in the mouth secretions of 10 out of 20 patients with open pulmonary tuberculosis.

There is no doubt that tubercle bacilli can be discharged in nasal exudate only infrequently; yet a detailed consideration of the possibilities of nasal infection is not unimportant in view of the finding by Hamburger, Green and Hamburger (1945) that bacteria are disseminated into the environment more readily and in much larger numbers from the nose than from the throat. Tuberculous lesions of the nasal cavity are rare, but a few cases have been reported, for instance of tuberculosis of the nasal septum (e.g. Renshaw, 1901). Tuberculous lesions are somewhat more common in the nasopharynx and it is quite possible that during a concurrent upper respiratory infection such as an acute coryza, some

tubercle bacilli may be carried forward through the nasal cavity and be discharged from the nostrils in the nasal exudate. Furthermore, it is possible that, apart from the existence of tuberculous lesions in the nasal cavity and nasopharynx, the nasal secretion may occasionally become contaminated with sputum coughed up from the lungs. Le Noir and Camus (1908) demonstrated the presence of tubercle bacilli in the noses of persons with pulmonary tuberculosis, but not in the noses of the physicians and attendants of the patients.

Early studies of nasopharyngeal tuberculosis have been reviewed by Lartigau and Nicoll (1902). "Adenoids", that is to say hyperplasia of the nasopharyngeal lymphoid tissue, has been observed to occur in a considerable proportion of otherwise healthy persons, especially children: in 1% of 1000 cases (Meyer), in 5% of 581 cases (Schmiegalow), in 7% of 650 cases (Wroblenski), in 9% of 15,000 cases (Rosenberg), and in 3% of 2000 school-children (Chappel). Tuberculous lesions have been demonstrated in the adenoids of a small proportion of such cases; for instance, Dieulafoy (1895) by animal inoculation demonstrated the occurrence of tuberculous infection of the adenoids in 7 out of 35 cases; Pluder and Fischer (1896) by histological examination found 5 of 32 adenoids to be tuberculous; Gourc (1897) using cultures, animal inoculations and histological examinations/

examinations did not find tuberculous infection of the adenoids in any of 201 cases; Lewin (1899) found tuberculous infection in 1 out of 20 adenoids examined by animal inoculations and in 9 out of 180 adenoids examined histologically; Lartigau and Nicoll (1902) by animal inoculation demonstrated the presence of tubercle bacilli in 12 out of 75 adenoids (16%), and by histological examination demonstrated tuberculous lesions in 8 of these 12 infected adenoids.

(7) Dissemination of Tubercle Bacilli into Environment in Sputum and Saliva.

From the various observations which have been detailed, it may be concluded that a majority of patients with manifest pulmonary tuberculosis discharge per day between 10 and 500 ml. of sputum containing from 100,000 to 10,000,000,000 tubercle bacilli. In the absence of hygienic precautions and domestic cleanliness, the greater part of this infected sputum is distributed on to floors and streets. In many cases, however, a spittoon or sputum-receptacle is used regularly, so that most of the discharged tubercle bacilli are disposed of in a manner not allowing opportunity for spread to other persons. Although use of a spittoon must reduce greatly the number of tubercle bacilli broadcast into the environment, it certainly does not prevent widespread distribution of small numbers of tubercle bacilli throughout the rooms occupied by patients. Heymann (1901) found tubercle bacilli in the dust of private rooms and hospital wards occupied by patients who used a spittoon. Chaussé (1914 c) demonstrated frequent development of tuberculosis in guinea-pigs which were kept in a multi-bed ward occupied by pulmonary-tuberculosis patients who expectorated into a spittoon containing phenol as a disinfectant; he also found tubercle bacilli in 7 out of 18 Petri dishes exposed to the air of a 2-bed ward occupied by patients/

patients who used a spittoon. In contrast, Brown, Petroff and Pesquera (1919-20) did not find tubercle bacilli in dust from floors, walls, bed-table, chair, telephone and carpet in Trudeau Sanatorium, and attributed this absence of infection to prophylactic disposal of sputum. At Olive View Sanatorium, Bogen and Dunn (1941) found tubercle bacilli in the air of admitting wards, but not in the air of wards containing only patients who had received instructions in hygienic behaviour. Smith, Urabec and Mason (1946) failed to find tubercle bacilli in the air of rooms at Barlow Sanatorium; they noted that this might reflect either success of the hygienic measures practised or inadequacy of the methods employed for finding tubercle bacilli.

Widespread distribution of tubercle bacilli occurring in spite of regular use of a spittoon, may be due in part to cough-spray or to distribution of saliva, but also to the distribution of important amounts of sputum which must inevitably escape the spittoon. Some sputum will be removed on the handkerchief, which, as noted by Koch (1884), even the cleanliest patient can not help soiling when wiping the lips after expectoration. In addition to such soiling of the handkerchief, Heymann (1901) noted that minute traces of sputum may be distributed on the lips, beard, hands, bed-linen and clothes, even when the patient/

patient is cleanly and a spittoon is used. Chaussé (1914 c) reported that the patients in the ward in which guinea-pigs were found to contract tuberculosis, possessed handkerchiefs which they used to wipe their lips and which they kept under their pillows; by direct inhalation-infection experiments with guinea-pigs, Chaussé demonstrated the presence of tubercle bacilli in 7 out of 10 handkerchiefs which had been used in the normal way by 7 patients.

Observations have already been quoted which indicate that a large proportion of persons with open pulmonary tuberculosis have tubercle bacilli present in their saliva, although in much smaller numbers than in their sputum (e.g. 100 to 100,000 times fewer; see Chaussé, 1914 a). Saliva may be distributed in many different ways, especially on the hands and on eating utensils. The hands frequently are allowed to touch moistened lips and frequently are used to wipe the lips; the fingers are sometimes licked or put into the mouth. Neild and Dunkley (1909) were struck by the freedom with which tuberculous patients use their saliva; they observed patients licking the finger to make it sticky enough to pick up a small object, and licking the finger to aid turning the leaves of a book. Neild and Dunkley also remarked on the common habits, among the general population, of licking envelopes, of licking a thread for threading a needle and of sucking pencils.

Kenwood and Dove (1915) provided a direct demonstration of the transfer of tubercle bacilli on fingers soiled with saliva. A small sheet of paper was given to each of 12 patients with open pulmonary tuberculosis. The patient moistened his thumb in his mouth and then smeared his thumb across the sheet of paper; this smearing was repeated 12 times. By guinea-pig inoculation, tubercle bacilli were demonstrated in the washings from 4 out of 6 sheets which were "washed" after standing dry under a bell-jar for 2 days following the smearing, and in the washings from 1 out of the remaining 6 sheets which were "washed" after standing for 1 month. Jacobs and Petroff (1941) inoculated animals with scrapings from pages of books which open pulmonary-tuberculosis patients had contaminated with their thumbs which had been moistened in their mouths; tubercle bacilli were not found in any case.

Saliva may be distributed on eating utensils such as spoons, forks, cups and tumblers. Cumming (1920), who regarded distribution of tubercle bacilli on eating utensils as the <u>major</u> avenue of distribution, considered the spoon to be the most likely utensil to act as a vehicle, since a large area of its surface is brought into contact with the mouth secretions. Tubercle bacilli have been found on spoons after their use by patients, by Cumming (1920), Rogers (1920) and Taylor (1921-22).

It is concluded that tubercle bacilli may be distributed in sputum expectorated on to the floor, and in sputum and saliva carried away on handkerchiefs, hands, eating utensils and other objects which are allowed to touch the lips, as sheets and pillow-cases, or are put into the mouth, as pencils.

(8) Dissemination of Tubercle Bacilli in Droplet Spray.

Droplets of secretion may be discharged from the respiratory tract by coughing, speaking, sneezing and spitting. It is generally considered, probably correctly, that droplets are not expelled in normal breathing. It must be noted, however, that the methods which have been used for examining normally-expired air for droplets and tubercle bacilli, rank as inefficient and inadequate by modern standards. Flügge (1899) stated that Cadéac and Malet, Grancher and Gennes, and Müller were unable to find tubercle bacilli in the ordinary expiration of phthisical patients. Koelzer (1903) examined 15 patients by having them breathe quietly for 7 to 15 minutes at a Petri dish held at 5 to 8 cm. in front of the mouth and then injecting 2 guinea-pigs with the contents of each of the 15 plates; only 1 out of the 30 guinea-pigs developed tuberculosis. Koelzer concluded that the quietly expired breath of consumptives is not entirely free from tubercle bacilli, but that tubercle bacilli are disseminated in the breath so seldom and to such a slight extent that the breath could not be of any practical importance as a source of infection. It should be noted that the single positive result obtained by Koelzer may have been due to infection of the exposed plate with airborne dust and not with the breath.

Among/

Among the spray-producing activities of consumptives, coughing has been considered the most important as it is especially frequent in occurrence. Koch (1884) noted that particles of sputum are discharged and scattered into the air by the patient's cough; he suggested that a healthy person might become infected by inhaling these particles, but thought that this would not occur commonly because "the particles of sputum are not small enough to remain suspended in the air for any length of time." Flügge (1897 a, b) showed that air could be infected by artificial atomisation of a bacterial suspension (B.prodigiosus); he suggested that the air in dwelling places might be infected naturally by the atomisation of saliva during coughing, speaking and sneezing. Later, Flügge (1899, 1901) suggested that pulmonary tuberculosis is transmitted mainly by the cough-spray of patients; on the basis of experiments performed by his pupils, Laschtschenko, Heymann, Sticher, Beninde and Möller, he argued that cough-spray was a much more important cause of infection than dried-sputum dust.

From the time of Flügge to the present day, numerous investigations have been made of the dissemination of tubercle bacilli in cough-spray. An effort has been made to learn not only the total numbers of tubercle bacilli and infected droplets which are expelled in coughing, but also the extent to which/

which tubercle bacilli are distributed in small droplets which are capable of remaining airborne for an appreciable time and are "respirable", that is capable of escaping the filtering action of the upper respiratory tract and of being drawn in inspired air through the whole length of the air passages to the alveoli of the lungs. In the first place, observations of the expulsion of tubercle bacilli in large projectile droplets will be reviewed, and then observations of the infection of air with small respirable droplets (droplet-nuclei).

The large "projectile droplets" and "settling droplets" of cough-spray are best demonstrated by microscopical examination after collection on glass slides, plates or Petri dishes which are held directly in front of the mouth during the bout of coughing, or are exposed facing upwards at greater distances in front of the coughing patient; sometimes the examinations are made by injecting guinea-pigs with washings from the exposed plates.

Laschtschenko (1899) exposed microscope slides around consumptives who were coughing; he found tubercle bacilli on 4 out of 21 slides (i.e. 19%).

Heymann (1899) exposed microscope slides on a table at 50 cm. in front of a coughing patient during a period of 2 hours; he found droplets containing tubercle bacilli in the case of 14 out of 35 patients (i.e. 40%).

Goldie/

Goldie (1899) found that in 14% of cases, tubercle bacilli were caught on plates after a single act of coughing; at one time or another, every patient examined gave positive results.

Ziesché (1907) exposed a 324-sq.cm. glass plate vertically at a distance of 40 to 80 cm. in front of the patient's mouth for a period of 30 minutes during which the patient coughed in his usual way; after staining by Ziehl's method, he examined the plate microscopically, counting the sputum and saliva droplets, and the tubercle bacilli within them. Ziesché reported 54 examinations made on 22 patients whose sputum contained tubercle bacilli; droplets containing tubercle bacilli were found to be expelled by coughing during the 30-minute period, by 8 of the 22 patients (i.e. 36%) on first examination and by a greater proportion of those examined on several occasions; in 29 of the 54 tests, tubercle bacilli were found on the exposed plates: less than 10 bacilli in 3 cases, between 10 and 100 bacilli in 15 cases, between 100 and 1000 bacilli in 9 cases, 1445 bacilli in 1 case, and 20,174 bacilli in 1 case.

Chaussé (1914 b) examined the cough-spray of 3 patients whose sputa were particularly rich in tubercle bacilli; during 30 to 40 natural coughs by the patient, he exposed at 15 cm. in front of the mouth a 234-sq.cm. glass plate which was attached to the/

the surface of a flat metal box filled with nearly boiling water. After staining, the plates were examined microscopically; salivary droplets were found on the plates, but tubercle bacilli were not found within them in any case. Chaussé's failure to demonstrate by this method the expulsion of tubercle bacilli in cough-spray is exceptional; however, it must be noted that he did not examine many patients.

By the same method as he used in the case of coughing, Chaussé found that during speaking a few droplets containing tubercle bacilli occasionally were expelled, but all of too large a size to be respirable. During spitting, on the other hand, when this was done explosively ("violent labial spit"), he found that a considerable number of droplets containing tubercle bacilli were expelled; for example, 5 out of a series of 22 droplets were infected, containing 21 tubercle bacilli altogether. However, Chaussé noted that consumptive patients usually spit gently rather than explosively, merely allowing the sputum to fall from the lips into the receptacle or handkerchief.

Chaussé (1914 b) made a further series of observations on cough-spray, using a different method. He examined 12 patients having 15,000,000 to 125,000,000 tubercle bacilli per ml. in their sputa. Petri dishes containing some sterile liquid were laid on a table at ½, 1, 1½ and 2 meters in front of the seated patient who coughed at them; the liquid from each/

each dish was injected into a guinea-pig. In all, the 12 patients coughed 1490 times at 33 dishes; only 2 of the 12 patients were found to expel tubercle bacilli, these being demonstrated in each case in a single dish exposed at ½ meter from the mouth.

Kenwood and Dove (1915) made observations on 15 patients suffering from advanced pulmonary tuberculosis with numerous tubercle bacilli in the sputum. A small sheet of paper was held 1 foot in front of the mouth at all times when the patient coughed during a 24-hour period; by guinea-pig injection with washings from the paper after standing dry for 2 days, tubercle bacilli were demonstrated on the papers of 8 out of 14 of the patients; there was not any proof that these papers were not infected by contact with the patient's fingers.

Rogers (1920) held a sterile Petri dish at 6 or 15 inches in front of the mouth of a patient who coughed at it 12 to 15 times, and then injected a guinea-pig with a saline washing of the dish; he demonstrated the expulsion of tubercle bacilli by 3 out of 4 patients tested at 6 inches, and by 3 out of 10 patients tested at 15 inches.

Hippke (1921) reported an extensive investigation on sanatorium and hospital patients suffering from open pulmonary tuberculosis. A frame holding three micrsocope slides (combined area about 55 sq. cm.) was placed/

placed at 25 to 30 cm. from the patient's mouth during coughing; the slides were stained by the Ziehl-Neelsen method and examined microscopically. Tubercle bacilli were found to be expelled on to the slides by 43 out of 587 patients (i.e. 7.3%) who gave a few voluntary coughs, by 35 out of 88 patients (i.e. 40%) who coughed naturally at the slides during 1 hour on each of 1 to 3 days, and by 61 out of 142 patients (i.e. 43%) who coughed naturally at the slides during 1 hour on each of 3 to 14 days.

Observations have been made of the morphology of infected cough droplets during microscopical examination of slides exposed to the coughing of patients and then stained. A droplet may consist mainly of sputum, mainly of saliva, or of an intermediate mixture of sputum and saliva. Tubercle bacilli are present most frequently and most abundantly in the sputum droplets.

Heymann (1899) described 3 types of droplets which contain tubercle bacilli: Type I, which is common and which consists of a central zone of fibrin threads, mucus, numerous leucocytes and a moderate or large number of tubercle bacilli, a middle layer containing mainly mouth epithelial cells and mouth commensal bacteria, but only a few tubercle bacilli, and an outer layer consisting largely of mucus and mouth commensal bacteria (e.g. staphylococci, streptococci/

streptococci and spirochaetes); Type II, which is rare and which consists almost entirely of sputum mucus flock corresponding to the central zone of Type I droplets, contains tubercle bacilli in moderate or large numbers (e.g. over 200 bacilli in a 500-micron droplet), and is enclosed by only a thin salivary layer containing a few mouth epithelial cells; and Type III, which usually is very small and consists of mucus containing a few mouth epithelial cells or leucocytes, and one or two isolated tubercle bacilli. In addition to these types of cough-droplets which contain tubercle bacilli, Heymann found in cough-spray droplets corresponding to all three types, which did not contain tubercle bacilli.

Ziesché (1907) found that most cough-droplets from consumptives belonged to one of two types, the salivary type which corresponded to Heymann's Type III, and the sputumous, or bronchial type, which corresponded to Heymann's Type II; the cough-spray of most patients contained larger numbers of salivary droplets than of bronchial droplets; the salivary droplets rarely contained tubercle bacilli, while the bronchial droplets frequently contained tubercle bacilli, often in large numbers.

Hippke (1921), like Ziesché, recognised two main types of cough droplets: mouth droplets (salivary) which contained mouth epithelial cells, numerous commensal mouth bacteria, but tubercle bacilli only rarely/

rarely and in very small numbers; and bronchial droplets (sputumous) which, in the pure form, contained only leucocytes, tubercle bacilli and other bacteria occurring in pure lung sputum.

Sim and Flinn (1939) exposed clean Petri dishes at 1 foot in front of the mouth during coughing; the dishes were stained for acid-fast organisms. Tubercle bacilli were seen to be contained in small specks of homogenous-staining albuminous material. These specks seemed very sticky and adherent, since mere washing did not suffice to loosen them from unstained cough dishes; positive animal inoculations with washings from the dishes were only achieved after scrubbing with a rubber-covered rod.

Early measurements of droplet size were made micrometrically on the dried and stained deposit marks of droplets caught on slides. Strauss (1926) showed that because of flattening of the droplets on the slide, the measured diameters of the droplet marks are about 2 or 3 times larger than the original diameters of the droplets while in the spherical state just before impingement. There is another cause of error in the case of droplets smaller than 100 microns in diameter which are collected on slides held at further than a few inches from the mouth; shrinkage due to evaporation while the droplet passes from mouth to slide, may be considerable in these cases.

Chaussé/

Chaussé (1913 a, b) was the first to show the relationship between the size of the droplet and the ability of the droplet both to remain suspended in the air and to be inhaled directly to the lungs. By measurement of droplet-marks on slides exposed in a small room at various times after artificial atomisation of an aqueous solution of dye, it was found by Chaussé (1913 a), and confirmed by Lange and Keschischian (1925), that all droplets giving deposit marks of 200 microns diameter and larger (i.e. of original spherical diameter of 70 to 100 microns, or larger, according to Strauss, 1926) fall out of the air within a few seconds, while smaller droplets, evaporating to dry particles of 2 to 20 microns, remain suspended in the air for periods of up to several hours. Wells (1934) confirmed this distinction from theoretical considerations; he calculated from basic physical data that droplets larger than 100 microns in diameter must fall to the floor within 3 seconds, while droplets smaller than 100 microns must evaporate at once to form minute "droplet nuclei" which can remain airborne for a long time. It may be noted that Chaussé (1913 a) found virulent tubercle bacilli to be present in the air of a small room for up to 7 hours after artificial atomisation of infective discharges.

By measurement of the "droplets" drawn through tubes by air currents of various speeds, it was found/

found by Chaussé (1913 b), and confirmed by Lange and Keschischian (1925), that only such "droplets" (droplet nuclei) as were less than about 20 microns in diameter were able to pass through bent and narrow tubes simulating bronchi, and thus would be capable of being inhaled directly into the lungs. By inhalation-infection experiments with rabbits, Wells, Ratcliffe and Crumb (1948) found that infected droplet-nuclei of 2 to 4 microns in average diameter penetrated almost quantitatively (i.e. 100%) to the lung alveoli, each giving rise to a tubercle, while of infected nuclei of 12 to 15 microns in average diameter less than 10% reached the lung alveoli and gave rise to tubercles. It must be noted that the latter observation does not prove penetration to the alveoli by a proportion (10%) of 12- to 15-micron nuclei; the 10% of nuclei reaching the alveoli may have been those less than about 5 microns in diameter constituting the lower extreme of a nucleus size-distribution spread widely about the average of 12 to 15 microns.

It may be concluded from these various observations that droplets with original diameters of more than 100 microns do not remain airborne for more than a few seconds, and, even if inhaled during this brief period of aerial flight, would be arrested in the upper respiratory tract and would not reach the lungs;/

lungs; and that droplets smaller than 100 microns evaporate at once to form droplet nuclei smaller than 20 microns, which may remain airborne for a few minutes or a few hours, and may be inhaled directly to the lung alveoli. In fact, if pulmonary tuberculosis is caused by direct implantation of tubercle bacilli in the lungs, it may be caused by droplets originally smaller than 100 microns in diameter, but it can not be caused by inhalation of larger droplets.

Chaussé (1914 b) and Hippke (1921) measured micrometrically the deposit marks of droplets caught on slides which were exposed in front of the mouth during coughing by consumptive and other patients; both investigators found that the "droplets" (droplet-marks) ranged from 30 to 3000 microns in diameter (i.e. from 15 to 1500 microns in original spherical diameter, according to Strauss, 1926). It is important to know with what frequency and in what numbers tubercle bacilli are present in those of the cough droplets which are smaller than 100 microns in original diameter.

Heymann (1899), examining microscopically slides which had been exposed at 50 cm. in front of the mouths of coughing consumptives, found tubercle bacilli in "droplets" (droplet-marks) down to 30 microns in diameter.

Chaussé (1914 b) examined the spray emitted by consumptives/

consumptives in projectile spitting ("violent labial spit"). The spray was caught on a glass plate held at 15 cm. in front of the mouth. This plate was kept at a temperature between 80 and 100 deg. C., so that after impingement the droplets were at once dried and fixed without alteration in size. After staining, the droplet-marks were examined microscopically for tubercle bacilli and their diameters were measured. The "droplets" (droplet-marks) ranged from 30 to 3000 microns in diameter; most were between 80 and 150 microns. Out of a series of 22 droplets, 5 contained tubercle bacilli: 1 bacillus each in a 60-micron, a 100-micron and a 150-micron droplet; 3 bacilli in a 140-micron droplet; and 15 bacilli in a 300-micron droplet.

Hippke (1921), using a microscope with an ocular micrometer, examined Ziehl-Neelsen stained slides which had been exposed at 25 to 30 cm. in front of the mouth of coughing patients. He found that tubercle bacilli were present in the majority, but not all, of the bronchial (sputumous) droplets. The smallest "droplets" (droplet-marks) which sometimes contained tubercle bacilli, usually only a single bacillus, were 15 to 20 microns in diameter. "Droplets" of 20 to 100 microns contained larger numbers of tubercle bacilli, even 15, 17 or 30 bacilli. "Droplets" of 100 to 500 microns contained usually less than 100 bacilli, but sometimes 250, 380, 684 and 845 bacilli. "Droplets"/

"Droplets" of 500 to 2000 microns contained frequently between 100 and 1000 bacilli, and rarely more: for example, 1497 in a 750-micron droplet, 7782 in a 825-micron droplet, 23,488 in a 1125-micron droplet, 4919 in a 1200-micron droplet, and 3075 in a 1325-micron droplet. Hippke found on slides exposed for several hours to the coughing of a patient who produced an unusually large number of small infected droplets, 39 "droplets" over 500 microns which contained altogether 16,428 tubercle bacilli, 306 "droplets" of 100 to 500 microns which contained 6892 tubercle bacilli, and 74 "droplets" under 100 microns which contained 232 tubercle bacilli. In a more typical case he found only 6 "droplets" over 500 microns which contained altogether 233 tubercle bacilli, and 7 "droplets" of 100 to 500 microns which contained altogether 103 tubercle bacilli.

It may be concluded from these measurements of Heymann, Chaussé and Hippke that the coughing of a consumptive sometimes expels a few droplets which both are less than 100 microns in diameter and contain some tubercle bacilli (between 1 and a few dozen). Thus, it appears definitely proven that the coughing of a consumptive will sometimes produce a few infected droplet-nuclei which are capable of being inhaled directly into the lungs of another person.

Direct proof that air may be infected by the coughing/

coughing of consumptives was sought by Laschtschenko (1899), Heymann (1899, 1901), Chaussé (1914 a, b;1916), Hippke (1921), and Sim and Flinn (1939). Patients with open pulmonary tuberculosis were made to cough within a room or small chamber, or into a box, and the air of this chamber was examined for the presence of suspended particles containing tubercle bacilli (i.e. infected droplet-nuclei). This examination was made either by recovery of the airborne particles by filtration of the air or by exposure of "settling plates" to the air, with subsequent injection of the recovered material into guinea-pigs, or, more simply, by exposure of guinea-pigs within the test chamber to breathe the contaminated air and so naturally contract pulmonary tuberculosis if the air should contain tubercle bacilli. Positive results in experiments of this kind must be interpreted with caution, since they may result from causes other than airborne droplet-nuclei. In the first place, the chamber air may become contaminated with infected dust-particles derived from the skin and clothing of the tuberculous patient giving the coughs; the various precautions against dustborne infection which were taken by the different investigators, were not in any case so rigorous and comprehensive as to preclude entirely the chance of error from this cause. The second way in which tests might yield positive results in the absence of droplet-nucleus air infection, is by means of/

of the large projectile droplets of cough-spray; if the air is examined by exposure of "settling plates" in front of or below the patient's mouth during the time of coughing as well as subsequently, then infected projectile droplets may be collected; if the air is examined by simple exposure of guinea-pigs, then tubercle bacilli in projectile droplets may contaminate the animals' fur and cage, and later be distributed into the air on infected dust particles and so be inhaled. Effective precautions against error from contamination by infected projectile droplets involve placing of the "settling plate" or guinea-pig away from the direct path of the cough-spray, or exposing of the "settling plate" or guinea-pig only at some seconds after cessation of coughing.

Laschtschenko (1899) carried out a series of experiments in a 3.2-cu.m. glass chamber. The patient's face and hands were first washed with some disinfectant, and he was clothed in a sterile coat and sterile shoes; these precautions against dustborne infection of the air might not be considered adequate by modern standards. The patient sat in the chamber for a period of 1 to 2 hours during which he coughed in his usual way. Several (5) Petri dishes containing saline solution lay exposed in the chamber during the period of occupation and coughing, some at head level and some above head level, at 90 to 170 cm. from the patient; the saline from these dishes was injected into/

into guinea-pigs. In 4 out of 9 such experiments, tubercle bacilli were found in some of the dishes. The positive results obtained with plates exposed above head level could have been due to infected dust-particles or to infected droplet-nuclei; the positive results obtained with plates exposed below head level could have been due to infected projectile droplets in addition.

Laschtschenko carried out another series of experiments by a method which excluded the possibility of contamination by projectile droplets. In tests with a patient coughing within, or into, the chamber during a period of 5 hours, a water pump or bellows was used to withdraw air from the chamber so that it bubbled through saline solution; the solution was then examined microscopically and by injection of guinea-pigs. The presence of tubercle bacilli in the chamber air was demonstrated in 0 out of 2 experiments in which 5 and 3 cu.m. were examined; in 1 out of 2 experiments in each of which 10 cu.m. were examined; and in 1 out of 3 experiments in each of which 10 cu.m. were examined and several patients coughed into the chamber instead of only one. Laschtschenko regarded these tests as proving that the coughing of a patient is immediately and by itself capable of infecting the air with tubercle bacilli; he noted that the amount of air infection was apparently slight, but suggested that/

that this infrequent finding of tubercle bacilli might be due to inefficiency of his air-filtration method.

Heymann (1899) made 6 experiments by exposure of guinea-pigs to the cough-spray of patients with open pulmonary tuberculosis. One of 4 different patients was used for each experiment. The patient's clothing was disinfected before each experiment, and the room used was disinfected repeatedly. A number of guinea-pigs were exposed at 25 to 45 cm. in front of the patient's mouth, either in a large common box or small individual boxes, which were open to the air. The patient coughed naturally at the guinea-pigs during a period of 1½ to 3 hours on each of several days (e.g. 14 days). Out of 25 guinea-pigs exposed to the coughing in the 6 experiments, 6 guinea-pigs (i.e. 24%) contracted tuberculosis, showing symptoms of inhalation tuberculosis. These experiments of Heymann, which appeared to prove that pulmonary tuberculosis is readily and frequently produced by exposure to air contaminated by the coughing of consumptive patients, were criticised by Chaussé (1916). Chaussé pointed out that Heymann's guinea-pigs were free to inhale not only airborne cough "droplets", but also airborne infected dust-particles derived from the patient's bed or person, and thus that Heymann had concluded <u>without reason</u> that infection/

infection was due to the "droplets"; moreover, Chaussé studied Heymann's protocols and doubted if the majority of the animals reported as tuberculous were so in fact. In addition to Chaussé's criticisms, it may be objected that Heymann's guinea-pigs were exposed also to infection by large projectile droplets.

Heymann (1901) made further experiments, in this case by the methods of Laschtschenko (1899), in an attempt to demonstrate the extent of infection of air by coughing. In a first series of experiments, a consumptive patient, who had removed his outer garment and put on a clean surgical gown, sat in a 3.2-cu.m. glass chamber for 1 to 1½ hours during which time he coughed naturally in his usual way. On a table in front of the patient, at a distance of 20 to 160 cm. from him, there lay uncovered 6 to 8 sterile Petri dishes; some dishes were also exposed behind the patient. The cough-spray was allowed to settle for several hours; the dishes were then washed with sterile broth and the washings were injected into guinea-pigs. With one or other of 2 patients, 6 experiments were made without a handkerchief being held in front of the mouth during the coughing; out of 34 dishes exposed, 24 dishes (i.e. 70.5%) collected tubercle bacilli from the air. A further 6 experiments were made with a handkerchief held in front of the mouth during the coughing; out of 36 dishes exposed, 11 dishes (i.e. 30.5%) collected tubercle/

tubercle bacilli from the air. In each of the 12 experiments at least one dish was positive. In 2 experiments a dish at 20 to 40 cm. behind the patient's back was positive. It might be concluded that while the majority of the positive dishes probably had been infected by large projectile droplets, the two positive dishes which were exposed behind the patient must have been infected by airborne droplet-nuclei. However, the precautions taken against dustborne infection from the patient's skin and clothing were not such as to preclude all possibility of error from this cause.

In a second series of experiments by Heymann (1901), a consumptive patient sat in the 3.2-cu.m. chamber for 1½ to 2 hours, coughing naturally. During the coughing, 12 Petri dishes lay on the table in front of the patient, covered by their lids. By the pulling of attached strings from outside the chamber, 4 of the dishes were uncovered at 15 minutes after the patient had departed from the chamber, a second 4 dishes at 30 minutes and the remaining 4 dishes at 90 minutes. The dishes were left uncovered and exposed to the chamber air for several hours; their contents were then injected into guinea-pigs. In this way, tubercle bacilli were demonstrated in only a single dish (of the 12 dishes exposed) in each of 2 out of 4 experiments. These positive dishes had not been uncovered until 30 minutes/

minutes after departure of the patient. Heymann concluded that the coughing of the consumptive had infected the air with tubercle-bacillus-containing "droplets" which remained airborne for more than 30 minutes after their expulsion.

In a third series of experiments by Heymann (1901), a consumptive patient sat in the 3.2-cu.m. chamber for 45 to 60 minutes and coughed naturally during this time. Air was aspirated from the chamber and passed through bottles of sterile water; the water then was centrifuged and the deposit injected into guinea-pigs, cultured or examined microscopically. A total of 7 experiments were made, each with 1 of 4 different patients. In 2 out of the 7 experiments, virulent tubercle bacilli were found in a 1-cu.m. sample of air taken while the patient sat coughing in the chamber. In none of the 7 experiments were tubercle bacilli found in 1-cu.m. or 2-cu.m. samples of air, the withdrawal of which was not begun until 10 or 60 minutes after the patient had vacated the chamber.

Bernheim (1905) was unable to infect animals with mouth-spray over 25 cm. from the mouth, but was able to collect tubercle bacilli on agar plates at the distance of a meter.

Chaussé (1914 a) investigated the readiness with which tuberculous sputum and saliva could be atomised artificially/

artificially to yield "respirable airborne particles" infective for guinea-pigs. An air jet with a velocity of 15 to 242 meters per second discharged upwards from just below the surface of the sputum or saliva in a cylindrical container ("deep ventilation"); in each experiment, 100 to 400 litres of the atomisation air passed from the container through bent tubes into a 126-litre chamber containing 6 to 10 guinea-pigs. Pulmonary tuberculosis, usually only a single lung lesion, was contracted by only 2 out of a total of 57 guinea-pigs exposed in 8 experiments with sputum ventilated at 15 to 36 m.p.s., by 3 out of 10 guinea-pigs in 1 experiment with sputum ventilated at 64 m.p.s., by 3 out of 8 guinea-pigs in 1 experiment with sputum ventilated at 85 m.p.s., and by 10 out of 10 guinea-pigs in 1 experiment with sputum ventilated at 242 m.p.s.; similar results were obtained with saliva. Chaussé (1914 b) presented evidence indicating that in coughing, speaking, singing and breathing, the maximum expiration air-speeds are much less than the air-speeds which were found in these experiments to be necessary for atomisation of sputum and saliva into infective respirable "droplets" (i.e. 64 m.p.s. and greater). He quoted his researches with Magne which indicated that during a violent cough the air-speed in the trachea was 5 to 17 m.p.s. and in the dilated glottis 7 to 25 m.p.s., and that in breathing, speaking and singing the air-speeds were 5 or 10 times less. Later researches by Chaussé/

Chaussé and Magne (1916) indicated that at the moment of coughing the air passing out through the opening glottis may attain the speed of 40 to 48 m.p.s.; this speed might be sufficient to atomise sputum from the vocal cords into fine respirable "droplets".

Chaussé (1914 b) also performed some experiments similar to those of Heymann (1899) in which guinea-pigs were exposed directly to the cough-spray of patients; Chaussé, however, arranged his experiments so as to minimise the chances of the guinea-pigs being infected by dust particles from the consumptive's clothing or by the large projectile droplets of his cough-spray. Patients were selected whose sputum contained very large numbers of tubercle bacilli. In each experiment one, or sometimes two, patients coughed many times (30 to 503 times) into a 86-litre inhalation box which contained a cage holding 9 guinea-pigs. The patient put his mouth near to and directed his cough-spray into a short 7-cm. diameter tube which opened into the inhalation box towards the top and one side. The cage containing the guinea-pigs was placed towards the other side of the inhalation box so as not to be in the direct line of the cough-spray; this obviated contamination by projectile droplets of the cage and the animals' fur, and so ensured that infection could be contracted only from airborne particles. Apart from the cough-spray introduction tube/

tube there was only one other opening in the inhalation box, a small decompression opening at the other side of the box. This confinement of the guinea-pigs in a box with only two small openings, reduced to a minimum the chance of the guinea-pigs acquiring tuberculosis by inhalation of dust particles from the skin and clothing of the patient outside; there remained only the small possibility of dust being blown into the box through the introduction tube by the blast of the coughing. In all, 8 experiments were made with 12 consumptive patients who gave a total of 1570 coughs; out of 79 guinea-pigs exposed in these experiments only 1 contracted tuberculosis, showing a primary lung lesion.

Chaussé (1916) made some further experiments in the same general plan, but with a smaller, 16-litre inhalation box; 7 to 12 guinea-pigs were exposed in a cage which almost filled the inhalation box. The patient coughed into a rubber tube which was 35 cm. in length and 3 cm. in diameter, and opened at its other end into the inhalation box towards the top of one side; the tube was slightly bent during use. As a cough expels 500 to 1000 c.c. of air, after 20 to 30 coughs the box must have been full of expired air. In each experiment the guinea-pigs were exposed in the box for 55 to 105 minutes to breathe the air contaminated by 52 to 316 coughs. In all, 18 experiments were made with 21 patients having from

80,000 to 106,000,000 tubercle bacilli per ml. in their sputum; these patients gave a total of 2901 coughs. Out of a total of 152 guinea-pigs exposed, 31 contracted tuberculosis; these infections occurred in 10 of the 18 experiments. Most of the negative results were obtained with the patients having fewest tubercle bacilli in their sputum. From these experiments and his other researches, Chaussé concluded that cough-spray from consumptives is capable of producing infection of the air with respirable particles containing tubercle bacilli, and thus can cause inhalation tuberculosis, but that the danger of such airborne infection by cough-spray is relatively slight; relatively few infections were caused in guinea-pigs exposed to a highly exaggerated concentration of cough-spray, as that of 30 to 503 coughs in the 86-litre box or of 52 to 316 coughs in the 16-litre box; infections of guinea-pigs were produced much more readily by dustborne infection of the air in other experiments which will be described later.

Hippke (1921) also carried out experiments by exposure of guinea-pigs to the coughing of consumptives. In a first series of experiments, the patient coughed through a glass tube, 15 cm. in length and 4½ cm. in diameter, into a 6.4-litre wooden box in which a guinea-pig was held with its nose/

nose at 22 cm. from the patient's mouth; following the coughing the box was shut and the guinea-pig was left in it for 1½ hours, after which time the guinea-pig was removed and kept out of rooms occupied by consumptives. None of the 5 guinea-pigs contracted tuberculosis in 5 experiments in each of which 10 to 19 coughs were given in 15 to 30 minutes by one of five patients who expelled only a few infected droplets on to exposed slides. All of the 10 guinea-pigs contracted tuberculosis in 10 experiments in each of which 8 to 60 coughs were given in 5 to 90 minutes by one of eight patients who expelled many infected droplets on to exposed slides. In a second series of experiments, the patient coughed at a guinea-pig held in a cylinder with its head projecting and freely exposed at 25 to 30 cm. from the patient's mouth. None of the 3 guinea-pigs contracted tuberculosis in 3 experiments in each of which 8 to 28 coughs were given in 30 to 45 minutes by one of three patients who expelled only a few infected droplets on to exposed slides. Only 1 of the 4 guinea-pigs contracted tuberculosis in 4 experiments in each of which 14 to 24 coughs were given in 45 to 90 minutes by one of four patients who expelled many infected droplets on to exposed slides. In a third series of experiments, the patient coughed at 6 guinea-pigs held in cylinders with their heads exposed at 35 to 40 cm. from the patient's mouth; 6 microscope slides were exposed beside/

beside the animals. None of the 6 guinea-pigs contracted tuberculosis in a first experiment in which 58 coughs were given during 60 minutes by a patient who at the same time expelled on to the 6 slides only 12 infected droplets containing a total of 331 tubercle bacilli. None of the 6 guinea-pigs contracted tuberculosis in a second experiment in which 64 coughs were given in 30 minutes by a patient who at the same time expelled many large infected droplets on to the 6 slides. All of the 6 guinea-pigs contracted tuberculosis in a third experiment in which 95 coughs were given in 60 minutes by a patient who in the latter half of this period expelled on to the 6 slides some 72 small infected bronchial droplets containing a total of 2394 tubercle bacilli and 9 larger infected droplets containing a total of 4408 tubercle bacilli. Having regard to the apparent dependence of the frequency of infection on the number of infected droplets observed to be expelled, it may be concluded that some of Hippke's guinea-pig infections were due to inhalation of infected droplet-nuclei produced by the coughing, though others were undoubtedly "contact infections" via the conjunctiva, pharynx or nose (see Lange, 1926).

Sim and Flinn (1939) arranged that a consumptive patient coughed into one compartment of a (9 x 9 x 15 in.) box which was divided in two parts by an/

an $\frac{1}{8}$ in. mesh screen; immediately after the coughing, 3 guinea-pigs were placed in the other compartment and left there for 1 hour. Two experiments were made, in the second of which the same 3 guinea-pigs were exposed on two occasions to the coughing of different patients. In each experiment 1 guinea-pig contracted pulmonary tuberculosis, while the other 2 guinea-pigs died early from an intercurrent infection.

It is concluded from the reviewed investigations that: (1) it has been established with certainty by microscopical examination of slides exposed in front of the mouth that the coughing of patients with open pulmonary tuberculosis usually expels many large projectile droplets which contain tubercle bacilli in small or large numbers; these infected projectile cough-droplets, being larger than 100 microns in diameter, can not remain airborne for more than a few seconds and can not be inhaled directly into the lungs; they may cause infection only indirectly, after impingement on skin, clothing, floor or furniture, by contact transmission to the mouth or by dustborne air infection.

and (2) it has been established by micrometric measurement of infected droplet-marks on slides exposed in front of the mouth that the coughing of patients with open pulmonary tuberculosis sometimes expels/

expels a few infected droplets which are smaller than 100 microns in diameter and which thus would be capable of remaining airborne as droplet nuclei and of being inhaled directly into the lung alveoli. The occasionally successful experiments in which guinea-pigs have been infected by exposure to the cough-spray of consumptives brings support to the view that the coughing of patients does sometimes infect the air with droplet nuclei containing tubercle bacilli and so causes inhalation tuberculosis; however, the chance of infection by dust or projectile droplets was not entirely precluded in such experiments.

(9) Survival of Tubercle Bacilli Outside the Body.

The likelihood of tubercle bacilli being spread by contact, eating utensils, fomites or dust, depends in large measure on the length of time for which the bacilli can remain alive and virulent under the environmental conditions of dryness and light.

Migneco (1895) found that when dried on cloth exposed to sunlight, tubercle bacilli lived for 20 to 30 hours.

Sticher (1899) found it difficult to demonstrate living bacilli in sputum dried and pulverised under natural conditions.

Heymann (1901) examined naturally-produced cough droplets from consumptives; these were collected and dried in dishes. The longest survival of the tubercle bacilli was 3 days in daylight and 18 days in the dark.

Hill (1902) dried sputum on glass rods in the air under ordinary room conditions; he found that all the bacilli were dead after 16 days.

Twitchell (1905) placed sputum in a folded handkerchief, in a folded carpet and as a smear on wood, and exposed it to the air at ordinary temperatures and in diffused daylight. The tubercle bacilli remained alive and virulent for guinea-pigs for/

for 39 to 70 days, but not for 110 days. In sunlight the bacilli died in a few hours.

Kirstein (1905) could not find any living tubercle bacilli after 8 days in artificially infected dust exposed to diffused light.

Cadéac (1905, 1908) was unable to reduce sputum to dust until it had been dried for 10 to 12 days, while the tubercle bacilli had nearly died out after the first 6 days. In sputum spread on marble, tubercle bacilli were all dead in 14 days.

Weinzirl (1907) found that tubercle bacilli would not survive 10 minutes in direct sunlight.

Rickards, Slack and Arms (1909) exposed sputum on wood and on cloth in the rooms of ordinary tenements. They found that when dry and exposed to diffused light, the tubercle bacilli lived for about 30 days; in dark and dry rooms, they lived for up to 85 days.

Chaussé (1912, 1913 e) found that the viability of tubercle bacilli in dried sputum in the conditions of a room was 10 to 25 days when tested by inhalation, and 30 to 60 days when tested by injection of guinea-pigs. Chaussé (1913 c, d) smeared cloths with tuberculous sputum, kept these for a time at room temperature and in diffused light, and then, by shaking or brushing, liberated dust from them into the air of a box containing 5 to 8 guinea-pigs; the occurrence/

occurrence of pulmonary tuberculosis in some of the guinea-pigs proved that tubercle bacilli survived for at least 16 days; the proportion of the exposed guinea-pigs contracting tuberculosis decreased steadily from the first to the sixteenth day. These experiments proved that tubercle bacilli survive drying in sputum on cloth for a time more than sufficient to allow pulverisation of the sputum and liberation of infective dust.

Kenwood and Dove (1915) showed by guinea-pig inoculation that tubercle bacilli survived for 2 days but not 31 days on sheets of paper which were contaminated with the cough-spray of consumptives and left folded to dry under a bell jar; in 1 out of 6 cases, tubercle bacilli survived for 31 days on a sheet of paper lightly smeared with tuberculous saliva.

Caldwell (1925) exposed tuberculous sputum mixed with sterile dust in open dishes out-of-doors; by guinea-pig inoculation she found that tubercle bacilli survived in sputum for at least 72 hours of exposure to direct sunlight (experienced during 21 days).

Lange (1926) dried tuberculous sputum in thin films on garnets and, after leaving these for different times at room temperature in daylight or in the dark, washed the garnets and injected guinea-pigs/

guinea-pigs with various, ten-fold dilutions of the washings. He found that some tubercle bacilli survived for at least 18 days, both in daylight and in the dark; however, the death rate of the bacilli was quicker in daylight, when it was 1000-fold in 2 to 6 days and 10,000-fold in 18 days, than in the dark, when it was 10-fold in 1 to 6 days and 100-fold in 18 days.

Smith (1942 a), because of repeated failure in attempts to find living tubercle bacilli in floor dust and on furniture in the immediate vicinity of patients at Barlow Sanatorium where the rooms are usually exposed to much daylight and sunlight unfiltered by glass, was led to suspect that the bactericidal effect of this light was largely responsible for the absence of infection. In experiments by culture and guinea-pig inoculation, he found that dried tubercle bacilli survived unfiltered north room-light for a minimum of 4 to 24 hours and a maximum of 5 to 12 days; in the dark, viability ranged from a minimum of 40 days to a maximum of 3½ to 5 months.

It is concluded from the investigations which have been reviewed that the human type of tubercle bacillus survives in dried sputum, saliva or cough-droplets at room temperature for between 2 weeks and 20 weeks in the dark, for between 3 days and 30 days in daylight filtered through glass, for between 4 hours and/

and 12 days in unfiltered daylight, and for between 10 minutes and 3 days in unfiltered sunlight. Clearly, these survival times are great enough to allow considerable opportunity for the spread of infection by contact, by eating utensils, by fomites or by dust.

(10) Occurrence of Tubercle Bacilli in Personal and Environmental Reservoirs of Infection: Namely, Lips, Hands, Clothes, Bedding, Handkerchiefs, Eating Utensils, Fomites, and Dust of Floor and Furniture.

The frequency with which living tubercle bacilli are present in various places in the environment of consumptives determines the likelihood of infection being spread by contact transmission to the mouth and by dustborne aerial transmission to the lungs. The occurrence of tubercle bacilli on lips, hands, eating utensils, door-knobs, books and other commonly handled objects, is especially important with regard to contact infection, while the occurrence of tubercle bacilli in clothes, bedding, handkerchiefs, floor dust and furniture dust, is especially important with regard to dustborne infection.

Tubercle Bacilli on Lips.

Gulbrandsen and Keller (1935) found tubercle bacilli on the lips of patients.

Tubercle Bacilli on Hands.

Baldwin (1898) found tubercle bacilli on the hands of patients in the Adirondack Sanatorium. Of 10 patients seen in private practice, 8 had tubercle bacilli on their hands.

Dieudonné (1901) by inoculation of guinea-pigs demonstrated tubercle bacilli on the hands of 2 out of 15 children.

Graziani/

Graziani (see Rosenow, 1908) found tubercle bacilli on the hands of 4 out of 8 tuberculous patients.

Ostermann (1908) washed the patient's hands in broth and injected guinea-pigs with portions of the washings. In this way he found tubercle bacilli on the hands of 7 out of 14 patients with pulmonary tuberculosis.

Cumming (1920) examined scrapings from the hands of tuberculous persons; the hands were soaked in warm water and the epithelium scraped off with a scalpel. Of 7 guinea-pigs injected, 3 became tuberculous.

Rogers (1920) had patients rinse their hands with sterile saline solution into sterile Petri dishes; by injection of guinea-pigs with the washings, he recovered tubercle bacilli from the hands of 2 out of 5 patients.

Tubercle Bacilli on Clothes.

Bissel (1899) by guinea-pig inoculation twice demonstrated tubercle bacilli in washings from the pockets of some uniforms which had been used by consumptives.

Friberger (1908-09) used a vacuum cleaner to remove dirt from clothing fresh from use by patients; he found virulent tubercle bacilli in 3 out of 12 tests.

Augustine/

Augustine (1929) removed dust by suction from the clothing of patients with open pulmonary tuberculosis. In each case, 6 cu. ft. of air was drawn from all articles of clothing except the handkerchief, especially from collars, lapels and button-holes; the air was passed through 10 ml. of water and half of this water was treated with sodium hydroxide and injected into guinea-pigs. In 6 out of 26 tests tubercle bacilli were found in dust from a patient's clothing.

Tubercle Bacilli on Bedding.

Rogers (1920) examined pillow-cases which had been used for 24 hours by bed patients with open pulmonary tuberculosis; each pillow-case was washed with sterile saline solution and the centrifuged deposit of the wash-water was injected into a guinea-pig. In this way the presence of tubercle bacilli was demonstrated on 2 out of 4 pillow-cases.

Tubercle Bacilli on Handkerchiefs.

Chaussé (1914 c) examined, after their normal use, the handkerchiefs of 7 patients with open pulmonary tuberculosis; after the handkerchief had been allowed to dry for 1 to 6 days, it was shaken in a box containing guinea-pigs; development of tuberculosis by the guinea-pigs indicated that tubercle bacilli were present on the handkerchiefs in 7 out of 10 experiments.

Tubercle/

Tubercle Bacilli on Eating Utensils.

Price (1908) inoculated 8 guinea-pigs with some water in which sanatorium dishes had been washed; all 8 guinea-pigs died of tuberculosis. On the other hand, washings from dishes which had first been washed in the ordinary way, did not infect guinea-pigs.

Cumming (1920) examined spoons immediately after their use at meals by patients with open pulmonary tuberculosis. Singly or in pairs the spoons from 42 patients were washed in 150-ml. amounts of hot water and the centrifuged deposit of 50 ml. of each wash-water was injected into a guinea-pig; of 31 guinea-pigs injected, 11 developed tuberculosis. In a further series of observations, 48 used spoons were first washed in the usual way in hot water and then, singly or in pairs, were rinsed for 5 minutes in 50 ml. of hot water; of 36 guinea-pigs injected with centrifuged deposits of the rinse-waters, 9 developed tuberculosis. Thus, Cumming found that a preliminary washing of the spoons "in the usual way in hot water" reduced the incidence of infection only from 35% to 25%.

Rogers (1920) also examined spoons immediately after their use at meals by patients with open pulmonary tuberculosis. The spoons were washed with 1% sodium hydroxide and guinea-pigs were injected with centrifuged/

centrifuged deposit of the washing after neutralisation. Out of 4 guinea-pigs injected, 3 developed tuberculosis.

Taylor (1921-22) reported examinations, which were made in two hospitals, of spoons immediately after their use at meals by patients with open pulmonary tuberculosis; some 42 spoons were examined while still unwashed and other 48 spoons were examined after "the usual thorough washing in hot water". A swab was rubbed over the bowls of 6 spoons and was soaked in 6 ml. of sterile saline solution; this solution was injected into 2 guinea-pigs. Of the 14 guinea-pigs injected from 42 unwashed spoons, 4 developed tuberculosis (i.e. 28.5%). Of 16 guinea-pigs injected from 48 washed spoons, none developed tuberculosis (i.e. 0%).

Thus, while all investigators found live tubercle bacilli on eating utensils immediately after their use by patients, Price and Taylor found that washing in hot water rendered the utensils non-infective, but Cumming found that washing in hot water failed to kill or remove all the bacilli.

Tubercle Bacilli on Fomites.

Mitulescu (1903) stated that the tubercle bacillus had been found in books after use for some years in a circulating library.

Kenwood and Dove (1915) examined 8 books just returned/

returned to a public library by patients with open pulmonary tuberculosis and 8 books in circulation in two tuberculosis sanatoria; dirty pages and covers were washed in saline solution and the centrifuged deposit of the washings from each book was injected into a guinea-pig. None of the 16 guinea-pigs developed tuberculosis (2 died early from other causes).

Rogers (1920) examined the covers of magazines which were much soiled by use in the open wards of a tuberculosis sanatorium, by washing them with 1% sodium hydroxide, neutralising, centrifuging and injecting the deposit into 3 guinea-pigs; 2 of the 3 guinea-pigs developed tuberculosis (the third died early from another cause).

Rogers (1920) also examined the door-knob of the door leading to the patients' dining-room at a tuberculosis sanatorium; the knob was handled by about 100 patients per day. The centrifuged deposit of saline washings from the knob was injected into 3 guinea-pigs; 1 of the 3 guinea-pigs developed tuberculosis.

Huhs (1906) found tubercle bacilli on napkin-rings at a sanatorium.

Klein (1908) by guinea-pig inoculation demonstrated the presence of tubercle bacilli in the combined/

combined swabbing from 6 telephones, while on another occasion (Klein, 1905) he did not find any tubercle bacilli on 12 telephones.

Brown (1921-22) reported that dust from the public telephone at Trudeau Sanatorium was examined by guinea-pig inoculation and that tubercle bacilli were not found.

Tubercle Bacilli in Dust of Furniture, Floors and Streets.

Cornet (1889) made the first extensive investigation of the occurrence of living virulent tubercle bacilli in dust in the environment of tuberculous persons. He used damp sponges to collect samples of dust from various surfaces, and injected each sample into 3 guinea-pigs. He took samples from city streets, and from walls, furniture and bedsteads in hospital wards, lunatic asylums, prisons, dispensaries and the private houses of consumptives. Altogether 147 dust samples were injected into 392 guinea-pigs; 59 of the guinea-pigs developed tuberculosis, 196 died early from other causes and 137 remained healthy; the positive results indicated that 40 of the 147 dust samples (27%) contained tubercle bacilli. Virulent tubercle bacilli were found in 0 out of 14 samples from streets, in 15 out of 27 samples from seven hospitals, in 3 out of 9 samples from asylums, in 21 out of 27 samples from the private rooms of patients whose sputum contained tubercle bacilli,/

bacilli, and in 0 out of 35 samples from the private rooms of patients whose sputum did not contain tubercle bacilli.

Schnirer (1891), unlike Cornet, was successful in demonstrating the presence of live tubercle bacilli in street dust.

Praussnitz (1891) found tubercle bacilli in 4 out of 10 samples of dust collected from coaches of a railway train much used by tuberculous persons.

Bissel (1895) demonstrated the presence of tubercle bacilli in the dust of trams.

Heymann (1901) collected samples of dust from hospital wards for consumptives and from the private sickrooms of consumptives. A spittoon was in use in all the hospital wards and in most of the private sickrooms. Samples were collected both inside and outside the "contact area" of the patient, that is the area within reach of his limbs and cough-spray (i.e. projectile droplets). Samples were taken from the floor, wall, skirting, bedstead, furniture, lamp-stand, cupboard-shelf, picture-frame, wall-clock and other fitments. Some samples were taken with a dry camel-hair paint brush which lifted only the lightest dust-particles, and other samples were taken with a wet sponge which lifted also the heavy particles unlikely to become airborne. By inoculation of guinea-pigs/

guinea-pigs with washings from the brush or sponge, the presence of tubercle bacilli was demonstrated in 44 out of a total of 239 dust samples (i.e. 18.4%) taken from the sickrooms of consumptives; the tubercle bacillus was found in one sample at least in 9 out of 20 different private sickrooms and in 15 out of 21 different hospital wards. The dust samples containing tubercle bacilli included 5 out of 59 samples taken with a paint-brush from private sickrooms (positive samples from 5 out of 13 rooms; 2 from inside and 3 from outside the "contact area"), 9 out of 57 samples taken with a wet sponge from private sickrooms (positive samples from 4 out of 13 rooms), 5 out of 61 samples taken with a paint-brush from hospital wards (positive samples in 5 out of 14 wards; 2 from inside and 3 from outside the "contact area"), and in 25 out of 62 samples taken with a wet sponge from hospital wards (positive samples from 11 out of 14 wards). From these observations of the widespread distribution of tubercle bacilli by patients who were cleanly and used a spittoon, Heymann concluded that the tubercle bacilli are distributed by infected dust derived from minute traces of sputum on the patient's mouth, beard, handkerchief, bed-linen and clothing. The recovery of tubercle bacilli in 10 out of 120 samples of dust which were collected with the dry paint-brush, proved that tubercle bacilli may be present in light dust particles/

particles which are easily capable of becoming airborne; tubercle bacilli were recovered rather more frequently with the wet sponge, in 34 out of 119 samples. The finding of tubercle bacilli as frequently outside as inside the patient's "contact area" indicated that the bacilli were distributed by airborne dust or droplet-nuclei rather than by contact or by projectile droplets.

Coats (1901) by Heymann's methods demonstrated living virulent tubercle bacilli in 66% of dust samples taken from 14 rooms occupied by tuberculous patients.

Hill (1902) found virulent tubercle bacilli in only 5 of 496 swabbings from private houses occupied by patients and in 3 of 180 swabbings from hospital wards.

Gotschlich (1903) did not find tubercle bacilli in any of 119 samples of dust taken from streets and public places.

Wagner (1903) by guinea-pig inoculation demonstrated tubercle bacilli in 3 out of 36 samples of dust taken from a sanatorium.

Köhlisch (1908) by injection of guinea-pigs demonstrated tubercle bacilli in 2 out of 15 samples of dust taken from the rooms of consumptives whose sputum contained numerous tubercle bacilli.

Chaussé/

Chaussé (1914 c) inoculated guinea-pigs with 11 samples of dust collected from various carriages of the Paris-Versailles train; he did not find tubercle bacilli in any.

Sweany and McLane (1919) in a Chicago municipal sanatorium found virulent tubercle bacilli in dust from 12 of 134 rooms occupied by patients with open pulmonary tuberculosis.

Brown, Petroff and Pesquera (1919-20) at the Trudeau Sanatorium did not find tubercle bacilli by guinea-pig inoculation of dust swabbed from the floors, walls, bed-tables and chairs of 2 single-bed rooms occupied by advanced cases of open pulmonary tuberculosis.

Augustine (1929) used a miniature vacuum cleaner to take samples of dust from the floor, rugs, matting, bed-frame, bureaux, chairs and other furnishings of the private rooms of tuberculous persons; each dust sample was mixed with saline and guinea-pigs were injected with untreated and alkali-treated portions of this mixture. Tubercle bacilli were found in 25% of the dust samples, in 6 out of 24 tests. Of 50 animals injected, 13 died early from pyogenic infections: 8 after injection of untreated dust and 5 after injection of alkali-treated dust.

Horwood (1931) on inoculation of guinea-pigs did not/

not find tubercle bacilli in any of 9 samples of dust, 3 of which were from private residences, 1 from an apartment house, 1 from a maternity ward, 1 from an office, 2 from college dormitories and 1 from a street; these places were not specified as being frequented by tuberculous persons.

Sim and Flinn (1939) inoculated guinea-pigs with dust from the floors of sanatorium wards and did not find any tubercle bacilli.

Smith (1942) uniformly failed to culture tubercle bacilli from swabbings from bed-side tables, lamps, bed-frames and other articles frequently handled in the rooms of open tuberculosis patients at Barlow Sanatorium.

From the results of these investigations, it may be concluded that tubercle bacilli frequently are present in the surface dusts of rooms occupied by persons with open pulmonary tuberculosis, but are present rarely if at all in rooms not regularly occupied by patients, and rarely if at all out-doors on streets.

(11) Transmission of Tubercle Bacilli by Contact and by Eating Utensils.

The possibility that pulmonary tuberculosis may be spread by contact is indicated by the demonstrations, just reviewed, of the presence of living tubercle bacilli on the lips, hands, clothes, bedding and handkerchiefs of patients, on eating utensils, books, door-knobs, telephones and other articles used by patients, and on floors and furniture in the dwellings of patients. Transmission of tubercle bacilli by direct or indirect contact, including transmission by eating utensils, leads to introduction of the bacilli into the mouth, and can hardly lead to inhalation of the bacilli to the lungs. Thus, evidence that tubercle bacilli are in fact transferred from patients to the hands and mouths of other persons is not in itself proof that pulmonary tuberculosis is spread in this way; proof is still required that tubercle bacilli taken into the mouth can cause primary infection of the lungs.

In order of intimacy of contact, the various transmission mechanisms are: (1) transmission by kissing, (2) transmission by putting into the mouth a spoon, fork, cup or other eating utensil after its use by a patient, particularly if the utensil is used without first being washed, but possibly even if used after washing, and (3) transmission by putting into the mouth the hand after its contamination by:

i) touching the patient, as in shaking hands,

ii)/

ii) touching an object just handled by the patient, such as a book, door-knob, telephone or chair-back, and iii) touching an article infected by the patient in a more indirect manner.

Brown (1921-22) described an experiment which showed how easily tubercle bacilli may be transferred by kissing. A consumptive patient kissed a glass plate; tubercle bacilli were found on this plate.

Cumming (1920) advanced the hypothesis that transmission of tubercle bacilli by eating utensils constitutes the main mode of spread of pulmonary tuberculosis; he suggested in consequence that the universal application of the principle of eating utensil asepsis will accomplish more in the control of tuberculosis than any other single measure of practical application.

The demonstrations by Price (1908), Cumming (1920), Rogers (1920) and Taylor (1921-22) that living tubercle bacilli frequently are present on spoons and other eating utensils immediately after their use by patients, have already been reviewed. There is no doubt that if such contaminated utensils were used by another person while still "dirty" and unwashed, living tubercle bacilli would be delivered into the mouth of the user. However, such use of "dirty" utensils can not occur commonly. Spread of infection by eating utensils/

utensils can only be of importance if the tubercle bacilli are able to survive the process of "washing". It appears that the temperature to which the bacilli are exposed in "washing with hot water", is sometimes sufficient and sometimes insufficient to kill them (Price, 1908; Cumming, 1920; Taylor, 1921-22).

Experiments have been made to show the extent to which tubercle bacilli are transferred by contact from a patient's hand to the hand of another person.

Graziani (see Rosenow, 1908) on several occasions found tubercle bacilli on his own hands after shaking hands with tuberculous patients.

Ostermann (1908) made preliminary experiments with the spores of a saprophytic bacillus. He contaminated the palm of his left hand with a suspension of the spores in sputum. After drying for 30 minutes, he pressed lightly the palm of his left hand against the thenar eminence of his right hand, as in hand-shaking. He then washed his right hand and made plate counts of the indicator organism in the washings. When the hands were not sweating, the highest number of bacilli recovered from the right hand was only 1 out of 22,000 of the number originally present on the left hand. When the hands were moist with sweat, the right hand yielded up to 1 out of 2700 of the number originally present on the left hand.

Ostermann (1908) also made experiments with tubercle/

tubercle bacilli on the hands, with 6 artificially infected hands and with 6 hands of unclean consumptive patients. The infecting hand was pressed firmly and rubbed on the hand to be infected. The latter was washed thoroughly in sterile broth and the broth was then injected intraperitoneally into guinea-pigs. Tubercle bacilli were found to be transferred from hand to hand in only 1 out of the 12 cases; this sole transfer of tubercle bacilli was from the hand of an unclean consumptive who suffered from sweating of the hands.

Brown (1921-22) selected two patients with abundant sputum containing numerous tubercle bacilli. These patients coughed violently on their hands. By inoculation of washings into guinea-pigs, the hands of both patients were proved to have become contaminated with tubercle bacilli. Nevertheless, tubercle bacilli were not found to be transferred from these contaminated hands to other hands as a result of hand-shaking.

A measure of the extent to which tubercle bacilli are transferred under natural conditions by direct or indirect contact from the patient to the hands of other persons, is given by examinations of the hands of healthy persons living in the environment of consumptive patients.

Ostermann (1908) by guinea-pig inoculation demonstrated/

demonstrated the presence of tubercle bacilli on the hands of 4 out of 42 children living in tuberculous families, and on the hands of a healthy nurse attending a consumptive patient.

Augustine (1929) rinsed the hands with a swab in 10 ml. of sterile water and injected this water into guinea-pigs. He did not find tubercle bacilli on the hands of any of 26 children living in 15 families having a tuberculous member.

From all these experiments and observations, it is apparent that tubercle bacilli can be transmitted by contact from a patient to the hands of another person, and thus presumably to the mouth of the latter. It is also apparent that tubercle bacilli are not transmitted by contact in this way either frequently or in large numbers.

(12) Transmission of Tubercle Bacilli by Flies.

If flies such as Musca domestica play any considerable part in the spread of tuberculosis, it will be by feeding on tubercular sputum and thereafter contaminating food or eating utensils. The possibility of spread by this means depends on whether pulmonary tuberculosis may result from the introduction of tubercle bacilli into the mouth. Another possible aid to transmission which may be provided by the activity of flies, is the distribution of tubercle bacilli from large, slow-drying sputum masses, to give numerous fine infected dust-particles wherever the fly faeces is deposited.

Flies are greatly attracted to sputum and will feed on it if possible. After feeding on sputum, the flies apparently suffer from diarrhoea.

Spillman and Haushalter (1887) were the first to suggest that house-flies fed on tubercular sputum might serve as carriers of infection. They found tubercle bacilli in the intestine and dejecta of flies fed on sputum.

Hofmann (1888) found tubercle bacilli in 2 out of 4 flies caught in a room occupied by a tuberculous patient, and also in flies' faeces scraped from the walls and furniture.

Lord (1904) placed about 30 flies under a jar together with a small dish of sputum showing about

10 tubercle bacilli per field, and around this some clean cover-glasses. Microscopical examination revealed that within 3 days the flies had deposited on the cover-glasses about 2000 specks containing each from 3000 to 5000 bacilli; that is, a total of about 100,000 bacilli were transferred per fly per day. By inoculation of guinea-pigs, the bacilli in specks protected from direct sunlight were proved viable and virulent up to the 15th day.

Hayward (1904) by inoculation of guinea-pigs demonstrated living tubercle bacilli in the faeces of flies after they had been fed on tubercular sputum through a fine wire screen so as to preclude infection of their feet and wings.

André (1908) demonstrated tubercle bacilli in the excreta of flies by animal inoculation; he found that the bacilli appeared in the faeces at about 6 hours after feeding and continued to be excreted for 5 days.

Graham-Smith (1910) by microscopical examination found that in flies fed on tubercular sputum, tubercle bacilli were carried in the intestine and excreted in the faeces for 4 to 7 days.

Brown (1921-22) described an experiment which showed that flies, although contaminated with tubercle bacilli, are not readily able to pass the bacilli to a host in such a way as to cause tuberculosis. A receptacle/

receptacle of sputum containing numerous tubercle bacilli was placed high up in a cage housing guinea-pigs. For a period of several weeks, 12 or more flies were kept in the cage, replacements being made for the flies which died. None of the guinea-pigs contracted tuberculosis as a result of this "natural exposure" for several weeks to flies having access to infective sputum.

(13) Contamination of Air with Dustborne Tubercle Bacilli.

The possibility that pulmonary tuberculosis may be spread by dustborne infection of the air is indicated by the demonstrations, already reviewed, of the frequent presence of living tubercle bacilli in dust on the clothes, bedding and handkerchiefs of patients, and on the floors and furniture of rooms occupied by patients. Furthermore, as will be detailed, it has been proved in many investigations that the disturbance of surfaces on which tubercular sputum has dried, readily distributes into the air dust-particles which carry tubercle bacilli; the presence of tubercle bacilli in the air has been demonstrated in some cases by injection of guinea-pigs with the material collected in "settling dishes" exposed to the air or in filters through which the air was drawn, and in other cases by direct exposure of guinea-pigs to breathe the polluted air and so contract tuberculosis by inhalation. Demonstrations of the latter kind in addition supplied proof that the airborne infected dust-particles are small enough to be inhaled directly to the lung alveoli.

The first demonstration of airborne infection with sputum dust was made by Tappeiner (1880) who found that tuberculosis could be produced in dogs by causing them to breathe dry and pulverised sputum.

Koch/

Koch (1884) thought that the air was more likely to become infected by dried sputum than by cough-droplets. He wrote: "Dried sputum, on the other hand, is much more likely to cause infection, as, owing to the negligence with which the expectoration of phthisical patients is treated, it must evidently enter the atmosphere in considerable quantity. The sputum is not only ejected directly on to the floor, there to dry up, to be pulverised and to rise again in the form of dust, but a good deal of it dries on bed-linen, articles of clothing, and especially on pocket handkerchiefs - which even the cleanliest of patients can not help soiling with the dangerous infective material when wiping the mouth after expectoration - and also is subsequently scattered as dust."

Cornet (1889) showed that the sweeping of a carpet covered with dried sputum caused infection of the air; tuberculosis was contracted by animals held in the clouds of dust raised by the sweeping. On the other hand, sputum dried in a mass was found more difficult to pulverise than sputum dried after smearing on a carpet. Hillier (1903) quotes Cornet as writing: "Anyone who has himself tried to rub the well-dried sputum into atoms and to pulverise it very finely will agree with me that it is no easy task to produce a really fine powder which remains suspended in the air for some time. The strong statements that have been made up to now - that one has only to rub with the foot on/

on the dried sputum to raise immediately a cloud of infectious germs - are absolutely false."

Cornet (1899) is quoted by Chapin (1912) as reporting the following experiment which proved that the air of a room may be heavily infected by the shaking of a sputum-soiled carpet. In a room of 76 cubic meters, 48 guinea-pigs were exposed in cages at various heights above the floor. Sputum was placed upon the carpet and after it was dry the carpet was shaken so that dust rose in clouds. This was repeated on 4 days. The result was that 47 of the 48 animals developed tuberculosis within 2 months. Kuss (1908) carried out similar experiments to this and obtained similar results.

Sticher (1899) carried out certain experiments which both he and Flügge (1899) interpreted as showing that airborne infection by dust does not occur readily, but which in fact showed that air is readily infected with dust by the rubbing of a sputum-soiled cloth, although not readily by blowing through an artificial mixture of dust and dried sputum. By powerful puffs from a rubber-ball hand pump, the air containing the dust was blown at 100 cm. per sec. into a confined inhalation space, the head bag of a guinea-pig which was exposed for 25 to 45 minutes. In 8 experiments dust was produced by rubbing muslin cloths which had been soaked in sputum and dried thoroughly/

thoroughly during 1 to 10 days, partly at 22 deg.C. and partly in a desiccator; 7 out of the 8 guinea-pigs contracted tuberculosis. In 3 experiments, air was blown through a small bottle containing an artificial mixture of dried sputum and fine dust; none of the 3 guinea-pigs contracted tuberculosis. In 5 further experiments, slower air currents were used, of 10 to 30 cm. per sec.; none of the 5 guinea-pigs contracted tuberculosis.

Beninde (1899) showed that handkerchiefs soiled with sputum and carried in the pocket for 24 hours afterwards, were not then dry enough to yield airborne infected particles when rubbed and exposed to strong currents of air.

Heymann (1901) infected the air in a 3.2-cu.m. glass chamber by beating carpets and shaking handkerchiefs; he observed the duration of air-carriage of the infected dust-particles. In 2 experiments, dust was raised by the beating of carpets which had been smeared with sputum and dried for 2 to 5 days. At 1.2 and 1.7 meters above the chamber floor a pair of Petri dishes were exposed during the dust-raising and other pairs of dishes for periods beginning at 10, 30, 45, 60, 90 and 120 minutes after the dust-raising. The contents of the dishes were injected into guinea-pigs. In both experiments tubercle bacilli were recovered from the air by the dishes exposed during the dust-raising, in both dishes in/

in one experiment and in one dish in the other. Tubercle bacilli were not recovered in either experiment in any of the 11 dishes exposed only after the end of the dust-raising.

In 3 similar experiments, Heymann (1901) raised dust by rubbing and shaking with the hand a handkerchief which had been smeared with sputum and dried for 2 to 5 days. In one of the experiments tubercle bacilli were recovered from the air only by a dish exposed during the dust-raising (by rubbing only). In each of the other two experiments tubercle bacilli were recovered by the dishes exposed during the dust-raising (by rubbing and shaking), but also by 3 of the 11 dishes which were exposed only after the end of the dust-raising, in dishes exposed after 15, 45 and 60 minutes, and 15, 30 and 30 minutes.

Heymann (1901) also made tests in which he examined the air by aspirating it through water and injecting the water into guinea-pigs. He thus demonstrated the presence of tubercle bacilli in the air on some occasions during sweeping of the sputum-soiled floor, during beating of a sputum-soiled carpet, and during rubbing of a sputum-soiled handkerchief; and also on one occasion at 30 minutes after sweeping the floor and on one occasion at 30 minutes after rubbing a handkerchief.

Köhlisch (1908) made 2 experiments in each of which/

which he exposed 9 guinea-pigs at various heights in a 3-cu.m. glass chamber. In the first experiment a wooden board on which sputum had been smeared, dried and mixed with street dust, was brushed with a hard broom within the chamber every 10 minutes during 2 hours' exposure of the animals. In the second experiment an old carpet which had been smeared with sputum and dried, was brushed with a broom. Tuberculosis, one or two lung lesions, was contracted by 15 out of 17 guinea-pigs from the two experiments.

Chaussé (1913 c, d; 1914 c) by direct exposure of guinea-pigs to breathe the air, demonstrated that air was readily infected by the brushing of soiled cloths and the shaking of soiled handkerchiefs. In a first series of experiments (Chaussé, 1913 c), 0.8 gm. of sputum containing numerous tubercle bacilli was put on a piece of pure wool cloth and allowed to dry for 2 to 16 days at room temperature in diffused light. The soiled cloth was then brushed for 10 minutes within a 126-litre metal box containing 5 to 8 guinea-pigs which remained in the box during the brushing and for about 3 hours subsequently. A total of 9 experiments were made with cloths dried for different times. Tuberculosis was contracted by all the guinea-pigs exposed to the brushing of a soiled cloth after 2 days drying, by all after 4 days, by 3 out of 6 after 6 days, by 1 out of 5 after 8 days, by 3 out of 6 after 10 days, by 4 out of 6 after 11 days, by/

by 4 out of 6 after 13 days, by none after 15 days, and by 1 out of 5 after 16 days; in all, about 30 out of 55 exposed guinea-pigs were infected.

In a second series of experiments made in a similar manner, Chaussé (1913 d) used handkerchiefs which had been smeared with about 1 gm. of sputum containing tubercle bacilli and had been dried under room conditions for 2 to 16 days. During 2 to 5 minutes the handkerchief was shaken from the end of a stick within the 126-litre box containing 5 to 8 guinea-pigs. Tuberculosis was contracted by 6 out of 6 guinea-pigs exposed to the shaking of a soiled handkerchief after 2 days drying, by 6 out of 6 after 4 days, by 6 out of 6 after 6 days, by 5 out of 5 after 8 days, by 7 out of 7 after 10 days, and by 1 out of 8 after 16 days. In a further 2 experiments with the naturally-soiled handkerchiefs of patients, after 1 and 2 days drying, 3 out of 6 and 6 out of 6 guinea-pigs contracted tuberculosis. In all, 40 out of the 50 exposed guinea-pigs were infected. The average number of primary lung lesions per animal in each experiment ranged from 1 to 26, being usually between 3 and 7.

In a third series of experiments made similarly, Chaussé (1914 c) used the naturally-soiled handkerchiefs of 7 highly infective patients. After drying for 1 to 6 days, the handkerchief was shaken 200 to 600 times in 2 minutes within the box containing

6/

6 to 10 guinea-pigs. The guinea-pigs were held in a cage inside the box so that nothing but dust from the handkerchief could reach them; they remained in the box for $1\frac{1}{2}$ to $2\frac{1}{2}$ hours. In 10 experiments infection of the air with dustborne tubercle bacilli was demonstrated 7 times; 41 out of a total of 73 guinea-pigs contracted tuberculosis.

In a special experiment, Chaussé (1914 c) demonstrated contamination of air with dustborne tubercle bacilli occurring under natural conditions. Two patients occupied a 2-bed ward at night only and spent the day in an adjacent room. On each of 70 days, 18 guinea-pigs in 3 cages were put into the ward for a short time while the patients were absent, at 14 hours after they had left in the morning, and while the ward was swept and the beds were made by healthy attendants. As a result of this exposure, 2 of the 18 guinea-pigs contracted tuberculosis, presumably by inhalation of infected dust-particles from the air.

Lange (1926) smeared a dirty woollen cloth with a thin layer of sputum containing a moderate number of tubercle bacilli, he allowed this to dry for 1 hour under room conditions, and then brushed it several times in a 246-litre glass chamber containing 4 guinea-pigs which were held in frames at 10 cm. above the chamber floor during the brushing and for 2 hours subsequently. All 4 guinea-pigs developed the typical primary tuberculous lesions of the lung.

In another series of 10 experiments, Lange (1926) contaminated woollen cloths with a coarse or fine artificial spray of sputum containing tubercle bacilli. After drying for about 24 hours the cloths were brushed or beaten for 30 seconds within the 246-litre chamber containing 2 to 4 guinea-pigs either free on the floor or held above the floor in frames. Primary tuberculous lesions of the lungs were developed by 9 out of a total of 38 guinea-pigs exposed; infections occurred under all the different experimental conditions tested, in 5 out of the 10 experiments.

Lange concluded that the sputum which is distributed on handkerchiefs, clothing and the floor in very small amount, as well as in droplet form, dries very quickly and so completely that the mechanical vibrations usual in daily life cause it to come off as dust. Of this dust a certain proportion always consists of very fine material which is capable of floating in the air. This portion appears to be rather large in the dust from linen and woollen materials, especially handkerchiefs.

Neufeld (1927) reviewing the work of Lange and others at the Koch Institute, emphasised that when sputum is smeared in a thin film on handkerchiefs or clothing, only a very brief period of drying, sometimes only a few seconds, is necessary before shaking/

shaking will liberate large amounts of infected dust. He noted that many of the bacteria-carrying dust particles are very small; in one case it was found that 8% of the bacteria put into the air by shaking a sputum-soiled handkerchief remained in the air for 1 hour.

(14) Occurrence of Tubercle Bacilli in Air under Natural Conditions.

Frequent demonstration of the presence of live tubercle bacilli in the air of rooms occupied by consumptives, has proved that airborne inhalation infection is capable of common occurrence. Such demonstrations of natural air infection have not distinguished the vehicle of the airborne tubercle bacilli, whether dust-particles or droplet-nuclei. Examination of the air has been made either by direct exposure of guinea-pigs to breathe the air, or by injection of guinea-pigs with material collected in "settling dishes" or in air-filtration devices. Positive results obtained with directly-exposed guinea-pigs are proof of air infection only if the tuberculosis contracted is identified at post-mortem examination as being of the primary pulmonary type; tuberculosis via the conjunctiva, nose or pharynx, might be caused by projectile-droplet or contact infection. Positive results obtained with "settling dishes" are not satisfactory proof of air infection unless these dishes are exposed in such a way, as above head level, that precludes the possibility of their contamination by projectile droplets and expectoration; unfortunately, some investigators did not take such precautions.

Le Noir and Camus (1908 a, b) demonstrated by inoculation the presence of tubercle bacilli in the dust/

dust of a hospital ward, but they could not recover tubercle bacilli from the ward air even by filtration of 53,000 litres.

Le Noir and Camus (1909) exposed guinea-pigs in a ward for phthisical patients. Four guinea-pigs were placed in a cage on the floor and the patients fed these; 1 of the 4 contracted tuberculosis. Five guinea-pigs were kept six weeks in a cage on the floor, but were protected so that the patients could not reach them; 1 of the 5 contracted tuberculosis. Four guinea-pigs were placed in a cage near the ceiling so that they had to be fed from a ladder; 2 of the 4 contracted tuberculosis.

Chapin (1912) made the following observations in the fairly clean house of a consumptive whose sputum contained large numbers of tubercle bacilli and who took no care with regard to its disposal. He exposed 36 small guinea-pigs in cages in a dark part of the room in which the patient usually sat; 16 of these animals were fed and cared for by the patient, and the other 20 animals by an employee of the Health Department free from disease. These latter animals were locked in a box covered with wire netting, 14 meshes per inch, so that contact with the patient was impossible. Exposure was from February 11th to May 14th. Of 10 surviving guinea-pigs tended by the consumptive, 7 contracted tuberculosis. Of 11 surviving/

surviving guinea-pigs exposed only to airborne and droplet-spray infection, 8 contracted tuberculosis.

Chaussé (1914 c) in 5 experiments at the Boucicaut Hospital examined the air of wards occupied by consumptives by keeping guinea-pigs in cages in the wards. In these wards, which were clean and well ventilated, no special precautions were taken with regard to sputum apart from use of a spittoon; each patient wiped his lips with a handkerchief which was kept under the pillow. When coughing the patients sprayed their bedding and clothing. The first experiment was made in a 1104-cu.m. common ward occupied by 32 male patients having from 1,000,000 to 160,000,000 tubercle bacilli per ml. in the sputum; during 34 days in the summer, 13 guinea-pigs were kept in a cage on a table in the centre of the ward; none of these 13 guinea-pigs contracted tuberculosis. The second experiment was made in a two-bed ward occupied by 2 patients having many tubercle bacilli in the sputum; during 34 days in the summer, 7 guinea-pigs were kept in each of two cages placed on the bed-side tables at 1 meter from the patient's mouth; only 1 of the 14 guinea-pigs contracted tuberculosis. The other three experiments were also made in two-bed wards; during 30 to 38 days in the winter and spring, 7 to 10 guinea-pigs were exposed in each of two cages placed between the beds of the patients at 2 to 2½ meters from their mouths; of the guinea-pigs exposed, some/

some 10 out of 16, 4 out of 14 and 15 out of 19 contracted tuberculosis. Thus, in the 5 experiments, tuberculosis was contracted by 30 out of 76 guinea-pigs exposed for about a month to the air of consumptive wards, by 1 of the 27 exposed in the summer and by 29 of the 49 exposed in the winter and spring. Of the 30 infected animals, 29 showed clear evidence that infection was of the primary pulmonary type. Since man inhales in a given period about 100 times more air than a guinea-pig, the infection rate of 30 out of 76 guinea-pigs per month indicates a high danger of airborne infection for man; a man living in the wards would have inhaled an infective particle within one day.

Chaussé (1914 c) also examined the air of a two-bed ward for consumptives by exposing 4 Petri dishes containing sterile water on the table, 4 dishes under the beds and 10 dishes on a holder at 80 cm. above the heads of the patients; development of tuberculosis by 13 of 50 guinea-pigs inoculated from these dishes proved that tubercle bacilli had been collected from the air by 7 of the 18 dishes.

Cumming (1920) drew 17,280 litres of air from a tuberculosis ward through water during each 24-hour test; the deposits from the wash-waters from 11 tests were injected into guinea-pigs and none of the 11 guinea-pigs developed tuberculosis.

Augustine/

Augustine (1929) examined the air in poorly ventilated private sickrooms of patients with open pulmonary tuberculosis who coughed frequently. Air measured by a gas meter was drawn through the "water aeroscope" of McConnel and Thomas (1925); the air was broken up into small bubbles by passage through a sheet of fine silk and then passed up through 25 ml. of distilled water in a cylinder. From each sample, some wash-water corresponding to 3.4 cu. ft. of air was injected into each of two guinea-pigs. In 24 experiments, 50 guinea-pigs were injected; only 1 of the 50 guinea-pigs developed tuberculosis, and this was shown to be due to the human-type tubercle bacillus; 6 animals died early from pyogenic infections. Thus, the tubercle bacillus was found once in about 170 cu. ft. of air.

Pressman (1937) and Eisenberg (1937) describe an investigation of air infection in four sanatoria. Petri dishes of 10 cm. diameter containing 20 ml. of normal saline were exposed in the corners of rooms, each for a period of 7 days. The attendants were instructed not to disturb the dishes. The centrifuged deposit of the liquid from each dish was examined by the microscope, by culture after treatment with oxalic acid and by injection of guinea-pigs after treatment. In all, 55 dish samples were collected from 15 rooms, including X-ray, pneumothorax, waiting, dining, private and children's rooms; 48 of the 55 samples/

samples were found to contain tubercle bacilli by microscopical examination, and all of the samples yielded tubercle bacilli on culture and on animal inoculation. Pressman (1937) did not recover tubercle bacilli in any 28-cu. ft. sample taken with the Wells air-centrifuge.

Sim and Flinn (1939) examined the air of wards at two sanatoria by exposure of "settling dishes" and by use of the air-centrifuge of Wells (1933). A total of 124 samples of air, each comprising 67½ cu. ft., were taken with the air-centrifuge from 12 wards, both during the morning sweeping and bed-making, and during the afternoon quiet. The samples were taken into 25 ml. of saline in the sampling tubes; after alkali treatment the saline was injected into guinea-pigs and also cultured. Tubercle bacilli were not found in any of the 124 samples comprising altogether 8370 cu. ft. of air. These negative results were attributed to inefficiency of the air-centrifuge when used with saline solution instead of agar, since tests with sprayed Strept. viridans also gave negative results with the saline. "Settling dishes", 800-ml. beakers containing 25 ml. of saline, were placed around the wards for periods of 7 days each; the patients were asked not to touch them or cough directly at them. Tubercle bacilli were not found in any sample on injection of guinea-pigs. It was not stated whether the dishes stood in light or shade.

Bogen/

Bogen and Dunn (1941) examined the air in rooms at Olive View Sanatorium by exposure of "settling dishes". Petri dishes containing 20 ml. of normal saline and 5% glycerol were left uncovered for periods of 7 days each. The alkali-treated deposit of the saline was examined microscopically, cultured and injected into guinea-pigs. Of 94 dishes exposed, 5 revealed a few tubercle bacilli on culture; 2 of the positive results were confirmed microscopically and 1 by guinea-pig inoculation. Of the positive results, 2 were obtained among 18 dishes exposed in the admitting wards, 3 among 35 dishes exposed in the laboratories, and none from wards containing only patients who had been instructed in hygienic behaviour.

Smith, Urabec and Mason (1946) uniformly failed to recover tubercle bacilli from the air at Barlow Sanatorium; they exposed 97 "settling dishes" for 7 days each in wards and other rooms, they used an electric pump to suck air through cotton-wool filters in 7 wards, and they used a device for drawing air through a nebulised spray of sterile water for about 6 hours in each of 5 wards; tubercle bacilli were not found when the collected material was examined by the microscope, by culture and by animal inoculation.

(15) Route of Entry of Bacilli Causing Pulmonary Tuberculosis: Introduction.

The majority of investigators, including Koch (1884), Cornet (1889) and Flügge (1899), took it for granted that pulmonary tuberculosis was caused by inhalation of airborne bacilli directly into the lung alveoli. However, Woodhead (1894), von Behring (1903) and Calmette and Guérin (1905; 1906 a, b) concluded from pathological observations and animal experiments that pulmonary tuberculosis is caused by ingestion of bacilli with their subsequent passage from tonsil or intestine via lymphatics to the lungs. Indeed, Calmette and Guérin argued that the air passages offer effective resistance to the entry of bacteria into the lungs in the inspired air, and that the immense majority of cases of pulmonary tuberculosis in man are caused by ingestion and not by inhalation of tubercle bacilli.

That commonly there is opportunity for tubercle bacilli to enter the body either by inhalation or by ingestion, is shown by the many demonstrations of the presence of living tubercle bacilli in air and dust, and on hands, eating utensils and fomites. The published observations of the occurrence of tubercle bacilli on these various vehicles do not indicate any marked difference between the opportunity for inhalation of bacilli and the opportunity for ingestion of bacilli. The relative frequency of infections by inhalation/

inhalation and infections by ingestion must be judged from other evidence: from observations as to whether it is physically possible for bacteria to pass in inspired air to the lung alveoli, to pass from tonsil to lung or to pass from intestine to lung; from comparative experiments on inhalation infection and ingestion infection of animals; from observations of the anatomical distribution of the primary-infection complex in human tuberculosis necropsies; and from observations of the relative frequency of occurrence of the human and bovine types of tubercle bacillus in pulmonary and other tuberculous lesions of man.

The importance of deciding this question, of how readily pulmonary tuberculosis may be caused by inhalation and how readily by ingestion, lies in the indication which an answer will provide as to the relative importance of airborne infection by dust and droplet-nuclei as against infection by contact, eating utensils and projectile cough-droplets.

(16) Possible Routes of Passage of Bacilli to Lungs.

i) Carriage in Inspired Air via Trachea to Lung Alveoli.

The available evidence shows beyond doubt that bacteria, if they are carried in small airborne particles, may pass directly in the inspired air to the lung alveoli, but, if they are carried on large particles, are filtered out in the upper respiratory tract.

Nenninger (1901) caused guinea-pigs and rabbits to inhale a fine spray of a B. prodigiosus suspension; he cultured portions of the respiratory tracts which were dissected out at 30 minutes after the inhalation, and he found B. prodigiosus in the smallest bronchi. Rabbits were caused to inhale dust containing B. megatherium spores and this organism was found in the lungs in nearly as great numbers as the spray-borne B. prodigiosus.

Calmette and Guérin (1905) in a few experiments with infected dust did not find that the bacilli reached the alveoli.

Hartl and Hermann (1905) showed that inhaled bacteria decreased in numbers very rapidly in passing back from the nose.

Bartel and Neumann (1906) after spraying guinea-pigs with tubercle bacilli found the germs immediately in the mouth, throat and lungs.

Kovacs/

Kovacs (1906) after inhalation experiments with tubercle bacilli could immediately recover them from the lungs.

Köhlisch (1908) made experiments with guinea-pigs to discover what fraction of the inhaled bacteria reach the lungs and are retained there. The heads of the guinea-pigs were exposed in an inhalation chamber for 10 to 60 minutes while dust impregnated with bacterial spores (e.g. 120,000,000 per gm.) was distributed through the air by the continuous blast from a propeller. The number of infected particles inhaled was calculated from the time of exposure, the animal's respiration volume per minute and the concentration of infected particles in the chamber air as measured by filtration and the making of plate counts. The number of infected particles reaching and retained in the lungs was estimated from plate counts made on broth in which immediately-dissected portions of the lungs were shredded. In 8 and 8 tests, the average proportion of the inhaled infected particles reaching and retained in the lungs was found to be 2.7% for cotton-spinning dust and 2% for floor dust. Köhlisch noted that Findel (1907) by a similar method found that on average 33% of inhaled infected droplets reached and were retained in the lungs.

Cobbett (1910) exposed guinea-pigs to a spray of B. prodigiosus, killed them within 5 minutes and by culture demonstrated the presence of B. prodigiosus in the/

the furthest parts of their lungs.

Chaussé (1913 b) by measurement of droplets produced by atomisation of a dye-solution, showed that "droplets" smaller than 25 microns in diameter were capable of being drawn in the least air current, as of 10 cm. per sec., or in a fast air current, as of 250 cm. per sec., through a glass tube with a dozen 60 deg. bends which simulated the bronchial tract; he presumed that such small particles could be inhaled to the alveoli of the lungs.

Similarly, Lange and Keschischian (1925) found that "droplets" smaller than 24, 20, 16 and 12 microns in diameter could be drawn at, respectively, 30, 100 150 and 400 cm. per sec., through a narrow spiral tube of 6 mm. internal diameter and with three 5-cm.-diameter spiral turns. Droplets of 20 to 100 microns could be drawn at these speeds only through a wider tube, of 10 mm. in diameter and with one right-angle bend; droplets of 100 to 700 microns could be drawn only through a straight tube which was 10 mm. in diameter and 20 cm. in length. In experiments with the heads of guinea-pigs exposed to the air of an inhalation chamber contaminated with dustborne or spray-borne bacterial spores, the number of spores inhaled was calculated from the exposure time, the guinea-pig's respiration volume per minute (330 cc.) and the concentration of spores in the chamber air as measured/

measured by concurrent sampling through saline and making dilution counts from this. The number of spores reaching and remaining in the lungs was estimated from dilution counts of saline washings of ground-up portions of the lungs dissected immediately after the exposure. When exposed to fine spray-droplets from a saline suspension of spores, on average 21.5% of the inhaled spores reached and remained in the guinea-pig's lungs. When exposed to fine airborne talcum-powder dust contaminated with spores, on average 12% of the inhaled spores reached and remained in the guinea-pig's lungs.

Hatch (1942) states from physical principles that particles larger than 5 microns are removed primarily by impingement in the upper respiratory tract, while particles smaller than 5 microns penetrate to the alveoli of the lungs and are deposited by settlement on the alveolar walls.

Wells and Lurie (1941) and Lurie and Abramson (1948), using the same technique, estimated the number of tubercle bacilli (contained singly in small droplet nuclei) which were inhaled by a rabbit, from the time of exposure, the rabbit's respiration volume per minute and the concentration of tubercle bacilli in the air as measured by sampling with an air-centrifuge; they estimated the number of tubercle bacilli reaching and remaining in the rabbit's lungs, by killing the animal/

animal immediately after exposure and making colony-counts from portions of its lungs. In all tests of exposure to small airborne infected droplet-nuclei, the number of tubercle bacilli collected in the lungs was found to be about equal to the number inhaled; that is, nearly 100% of the inhaled infected particles penetrated to and remained in the lungs.

It is concluded that nearly 100% of small infected particles inhaled (under 5 microns) reach the lungs, while of inhaled dusts of various kinds, only from 2% to 12% of the infected particles are small enough to reach the lungs.

ii) Passage by Lymphatics from Tonsils to Lungs.

Some evidence has been advanced to suggest that bacilli may pass from the tonsils to the lungs via the cervical and mediastinal lymphatics, or via the submucosal lymphatics of the trachea.

Grober (1905) injected large quantities (10 ml.) of India ink into the tonsils of dogs and found that the ink passed down the neck, by what he thought to be a lymphatic route, to the apical pleura, lungs and bronchial glands. Wood (1906) and Cobbett (1917) denied the existence of such a connection, the latter author suggesting that owing to the huge volume injected, the ink was forced mechanically through the loose connective tissue of the neck.

Wintermitz, Smith and Robinson (1920) stated that there/

there is a rich plexus of valveless lymphatics within the submucosa of the trachea and bronchi, and that this may supply a pathway for invasion of the lungs from the throat. India ink injected into this plexus spread up to the epiglottis and down to the smaller cartilage-bearing bronchi, where there exist anastomoses with peribronchial and periarteriolar lymphatics leading to the lung substance.

iii) Passage by Lymphatics from Intestine to Lungs.

Much evidence has been presented to suggest that bacilli may pass from the stomach or intestine to the lungs via the mesenteric and mediastinal lymphatics.

Woodhead (1904) stated that at post-mortem examination the course of invasion often could be traced from intestine to lungs by a continuous chain of tuberculous lesions in the mesenteric, retroperitoneal and mediastinal glands.

Van Steenberghe and Grysez (1905) and Whitla (1908) introduced Chinese ink into the stomach of adult guinea-pigs by an oesophageal tube, and found at 4 to 24 hours later that the lungs were pigmented with the carbon, although the mesenteric glands were not pigmented. Cobbett (1910) was unable to confirm these findings; he found a degree of pulmonary anthracosis in animals not fed the ink, and suggested that the previous investigators had erred through not examining control animals.

Schlossman/

Schlossman and Engel (1906) injected tubercle bacilli into the stomachs of young guinea-pigs through an incision in the abdominal wall; they found tubercle bacilli in the lungs on dissection at 6 hours later.

Ravenel and Reichel (1908) in similarly conducted experiments found tubercle bacilli in the lungs of 28 out of 50 young guinea-pigs within 24 hours after inoculation of the stomach; it was thought that the tubercle bacilli had been absorbed from the intestine to the lungs, but it was noticed in all of 10 guinea-pigs sacrificed only after some weeks that infection of omentum and abdominal wall had been caused by the inoculation, so providing another possible source of bacilli.

Griffith (1907) fed 12 dogs with large amounts of tubercle bacillus culture; he killed the dogs after several hours and injected their shredded mesenteric glands into guinea-pigs; he found tubercle bacilli in the mesenteric glands of 6 of the 12 dogs. Tubercle bacilli were not found in the thoracic-duct chyle of any of 11 dogs. Griffith (1911) fed large doses of tubercle bacillus culture to 9 pigs, 3 monkeys, a goat and a cat, killed the animals at 2 to 13 days afterwards and found tubercle bacilli in the mesenteric glands of all the animals except one monkey, and in the lungs of 4 pigs, 1 monkey, the goat and the cat.

Cobbett/

Cobbett (1910) on some occasions was able to demonstrate the presence of tubercle bacilli in the lungs of rabbits and guinea-pigs at 17 to 42 hours after feeding with culture.

Cobbett (1917) reviewed these observations just described, and concluded that tubercle bacilli do occasionally pass within a short time through intact mesenteric or cervical glands when animals are fed very large numbers of bacilli. However, he pointed out a possible fallacy in such demonstrations; the tubercle bacilli may pass from the stomach to the lungs by regurgitation and aspiration, instead of by the lymphatics.

iv) Passage from Mouth to Lungs by Aspiration down the Trachea.

Cobbett (1917) described experiments in which B. prodigiosus was given to animals by mouth in a variety of ways; he found that even when the animal was killed immediately, before any absorption from the intestine could occur, B. prodigiosus invariably had reached the lungs, presumably by aspiration via the trachea.

It should be noted that such aspiration might occur either by a mass of infective secretion from the mouth in some way escaping through the glottis and being drawn bodily down the trachea, or, as appears more likely, by infective secretion in the mouth being atomised by the respiratory activities of the animal and the small autogenous droplets being inhaled.

(17) Experimental Induction of Tuberculosis in Animals.

At an early date it was shown that pulmonary tuberculosis readily could be induced in animals by inhalation of tubercle bacilli, both in sputum-dust (Tappeiner, 1880) and in droplets of sprayed sputum or culture (Koch, 1884). Later, the induction of pulmonary tuberculosis in animals by administration of tubercle bacilli into the alimentary tract, by ingestion, was claimed by Calmette and Guérin (1905; 1906 a, b). However, it has been found in most subsequent investigations that for induction of tuberculosis of any kind, pulmonary or alimentary, much larger doses of tubercle bacilli are required when given by ingestion than when given by inhalation; small doses induce tuberculosis in a large majority of animals when given by inhalation, but only in a small minority when given by ingestion.

i) Experimental Comparison of Facility of Inhalation-Infection and Ingestion-Infection in Animals.

Koch (1884) made various experiments showing how easily animals contract pulmonary tuberculosis by inhalation of airborne infected particles. He gave an account of Experiment 26 as follows: "A very roomy box, having on one side an opening for the orifice of the spray apparatus, was placed in a garden at a good distance from any habitation. The spray apparatus was placed outside the box, with its orifice projecting into the interior. The apparatus was connected by means of elastic tubing and a suitable length/

length of lead pipe, which passed through the woodwork of a closed window, with the India rubber bellows, and so could be worked from the room beyond the region of the spray.

Pure culture from a phthisical lung in the human subject (No. 1, carried through twenty-three generations in fifteen months) was rubbed with distilled water, and the fluid diluted to such an extent that it looked almost clear. Any visible fragments present in the fluid subsided after standing a short time; the upper layer, which showed hardly any opacity, was poured off and used for inhalation. Fifty cubic centimeters were dispersed in the course of half an hour on three successive days, and inhaled by the animals in the box as follows: 8 rabbits, 10 guinea-pigs, 4 rats and 4 mice. After the inhalation the animals were kept in separate rooms in cages, and were well looked after. In some of the animals dyspnoea appeared after ten days, and 3 rabbits and 4 guinea-pigs died in the course of fourteen to twenty-five days. All the remaining animals were killed at twenty-eight days after the last inhalation. All the rabbits and guinea-pigs had numerous tubercles in the lungs, the size of the tubercles being proportionate to the length of time the animals had lived after the inhalation."

Gebhardt (1890), working with diluted sputum, found that 800 tubercle bacilli sufficed to infect guinea-pigs/

guinea-pigs when administered as a spray in the inhaled air, while 10,000,000 failed to do so when swallowed.

Preyss (1891) was able to infect guinea-pigs by inhalation with only 40 bacilli.

Kossel, Weber and Heuss (1905) found that 1 mg. of tubercle bacillus culture would infect calves by inhalation, while 1000 mg. by feeding produced little more than minimal lesions.

Weber and Titze (1910) found that 0.01 mg. of culture would infect a calf by inhalation, while 10 mg. of culture were required to infect a calf by feeding.

Findel (1907) found that inhalation of 62 tubercle bacilli regularly infected adult guinea-pigs, while doses of up to 382,000 bacilli given by feeding failed to cause infection. A suspension of tubercle bacilli of the required concentration was made, taking 1 mg. of moist culture as containing 35,000,000 living bacilli (c.f. 46,000,000 per mg., Wilson and Schwabacher, 1937). This suspension was sprayed as a fine mist into the bottom of a cylinder, 30 cm. in height and 15 cm. in diameter, which was aspirated from the top. In each test 4 guinea-pigs were held with their heads projecting through ports into the cylinder. The dose of tubercle bacilli inhaled was calculated/

calculated from the time of exposure and the animal's respiration volume per minute (i.e. 333 cc./min. for a 325-gm. guinea-pig, and 357 cc./min. for a 625-gm. guinea-pig). Inhalation tests were made with a total of 83 adult guinea-pigs which breathed doses of 20 to 290,000 bacilli. Severe tuberculosis was contracted by all the guinea-pigs which inhaled doses of 62 bacilli or more, by 1 of 3 guinea-pigs which inhaled 40 bacilli, and by 2 of 3 guinea-pigs which inhaled 20 bacilli; the two infected animals inhaling 20 bacilli showed in the lungs, respectively, 1 and 2 millet-seed nodules which were identified microscopically as tubercles. In all infected guinea-pigs which were examined after 28 days, the tubercles were almost entirely situated in the lungs and bronchial glands; after 50 days, the tuberculosis had spread to all organs. Feeding tests were made with 14 guinea-pigs which ingested doses of 19,000 to 382,000 tubercle bacilli; none of these showed evidence of tuberculosis on careful examination up to the 174th day. Further experiments suggested that the minimum infective dose by feeding was 10 mg., about 350,000,000 bacilli, or 6,000,000 times the lethal inhalation dose.

Findel was able to infect dogs by inhalation with doses down to 0.14 mg., while feeding with doses up to 63 mg. produced no effect whatever.

Reichenbach/

Reichenbach (1908) exposed animals in a box into which was sprayed by a Buchner spray a measured suspension of tubercle bacilli (human-type tubercle bacillus culture assumed to contain 40,000,000 living bacilli per mg.). Guinea-pigs were found to be infected by inhalation when 40,000 bacilli were sprayed, but not when 4000 were sprayed (of which only a proportion would be inhaled). Guinea-pigs were infected by feeding with doses of 140,000,000 to 2,000,000,000 bacilli, but not with doses of 14,000,000 bacilli or fewer.

Reichenbach found that goats could be infected by inhalation with 0.01 mg. of culture, while 5 mg. by ingestion only produced minimal lesions.

Köhlisch (1908) in experiments with guinea-pigs exposed to inhale dust impregnated with a known number of bacterial spores per gram, found by immediate dissection that the average proportion of the inhaled infected particles which reached and was retained in the lungs, was 2.7% for cotton-spinning dust and 2% for floor dust. In subsequent experiments the dust was impregnated with sputum containing a known number of tubercle bacilli, as well as being impregnated with the spores. Without the need for immediate sacrifice and post-mortem dissection, the number of tubercle bacilli reaching the lungs could be calculated from the number of spores demonstrated to be present in the inhalation-chamber air by filtration. The exposed guinea-pigs were/

were observed for development of tuberculosis. Tuberculosis was produced by inhalation of infected cotton-spinning dust in tests in which 700 tubercle bacilli reached the lungs, but not in tests in which only 450 and 600 bacilli reached the lungs. Tuberculosis was produced by inhalation of floor dust in tests in which 300, 2600, 2900 and 3500 bacilli reached the lungs, but not in tests in which 70, 300, 500, 600, 2000 and 2500 bacilli reached the lungs. In experiments with sprayed sputum, tuberculosis was caused by all doses down to the smallest, namely 50 bacilli, which gave rise to over 20 tubercles in the lungs.

Lange (1924) found that tuberculosis was contracted by a small minority of guinea-pigs which ingested very small numbers of tubercle bacilli. He assumed that 1 mg. of culture contained about 100,000,000 living bacilli. One or two drops of bacillary suspension were dropped into the mouth while the animal's head was held in a natural position so as to avoid any aspiration to the lungs. A single dose of 0.000,000,1 mg. to 1 mg. was given by mouth to each of 60 guinea-pigs; 17 of the 60 animals contracted tuberculosis; 64% of those receiving 0.1 mg., 50% of those receiving 0.001 mg. and 25% of those receiving 0.000,01 mg. (3 out of 22 receiving 0.000,01 mg. or less). The smallest dose found to be effective on some occasions was 0.000,000,1 mg., or about 10 bacilli,

after/

after feeding 12 times.

Lange and Nowoselsky (1925) counted the primary tuberculous lesions induced in the lungs of guinea-pigs inhaling minimal numbers of droplet-borne or dustborne tubercle bacilli of the bovine type. The heads of the guinea-pigs were exposed in an inhalation chamber contaminated by air-blasts from a pump distributing fine talcum powder in which dried culture had been finely ground, or else the fine droplets of an artificially sprayed saline suspension of culture. The number of bacilli inhaled by the guinea-pig was estimated from the exposure time, the animal's respiration volume per minute (330 cc.) and the concentration of bacilli in the chamber air as measured by concurrent sampling through saline, dilutions of which were examined by injection of other guinea-pigs. From these numbers inhaled, the numbers reaching and remaining in the lungs were calculated according to the ratios determined by Lange and Keschischian (1925), namely 12% and 21.5% for talcum dust and fine droplets respectively. In three experiments the calculated numbers of tubercle bacilli collected in the lungs were confirmed by killing the animals immediately after the inhalation and injecting portions of their lungs into other guinea-pigs. In 6 experiments with infected talcum "dust", a total of 13 guinea-pigs were exposed and each was calculated to receive into its lungs between

1/

1 and 20 tubercle bacilli, on average about 4; 8 of the 13 guinea-pigs became infected, each developing from 1 to 5 primary tuberculous lesions in the lungs. In an experiment with culture spray, each of 2 guinea-pigs was calculated to receive 36 to 360 bacilli into its lungs; each of the 2 animals became infected, developing respectively 100 and 130 tubercles in the lungs. In a second experiment with the spray, each of 2 guinea-pigs was calculated to receive 14 to 72 bacilli into its lungs; each of the 2 animals became infected, developing respectively 13 and 16 tubercles in the lungs. It was concluded that a single dust-borne or droplet-borne tubercle bacillus inhaled into the lungs is capable of inducing the formation of a tuberculous lesion. In all cases the infection was a primary infection of the lungs.

Wells and Lurie (1941) described an apparatus for quantitative inhalation infection of rabbits. A suspension of a highly virulent bovine-type tubercle bacillus was atomised and the droplet-nuclei so formed were drawn into an inhalation chamber. The number of bacilli inhaled was calculated from the exposure time, the rabbit's respiration volume per minute (estimated as 500 cc.), and the concentration of tubercle bacilli in the chamber air as measured by concurrent sampling with a Wells air-centrifuge into liquid from which colony counts were made on solid egg-medium. Experiments were made on rabbits from inbred/

inbred families, some of high and some of low genetic resistance to tuberculosis. Pulmonary tuberculosis was contracted by all of 17 rabbits of various families, following inhalation of doses of from 23 to 6800 bacilli; from the lowest to the highest dose, the time until development of hypersensitivity was reduced from 4-8 weeks to 2-3 weeks, the time until death reduced from 16-25 weeks to 5-7 weeks, and the number of primary caseous pneumonic nodules increased in almost equal numbers with the inhaled bacilli. With inhaled doses of more than 1000 bacilli, it made little difference whether the rabbit belonged to a resistant or susceptible family. With doses of less than 100 bacilli, rabbits from susceptible families developed a disease soon spreading from the primary foci in the lung to the draining lymph glands and to the other organs of the body, and causing a fairly early death, as at 16 weeks (i.e. a disease resembling the progressive first infection of childhood in humans); while rabbits from resistant families developed a disease remaining localised to the point of origin in the lung, giving rise to large cavities and causing relatively late death, as at 25 weeks, without involvement of the lymph glands or other organs (i.e. a disease resembling the chronic re-infection type of pulmonary tuberculosis in human adults).

Wells/

Wells (1948) described an improved apparatus for the quantitative study of droplet-nucleus infection of animals; 6 rabbits could be exposed simultaneously to the air in a central inhalation chamber.

Using this apparatus, Wells, Ratcliffe and Crumb (1948) counted the number of tubercles developing in the lungs of normal rabbits following inhalation of different numbers of large or small droplet-nuclei containing single tubercle bacilli of a virulent bovine strain. The tubercle bacilli were grown in broth in a rotating flask containing large glass beads; the culture was filtered through filter paper to yield a suspension consisting mainly of singled bacilli. A suspension of 1,000,000 bacilli per ml. was atomised to give about 200,000,000 droplets per ml., of about 18 microns average diameter, and thus to give infected nuclei mainly containing each a single bacillus. The average size of the droplet-nuclei was determined by the concentration of Difco brain-heart infusion solids which was dissolved in the suspension to be atomised; the average nucleus diameter was about 2 microns when the infusion concentration was under 0.1%, 4.4 microns when 1%, 8.7 microns when 10%, 12.0 microns when 33.3%, and 15.1 microns when 66.6%. The nucleus diameter measurements were estimated from average settling velocities which in turn were calculated from parallel "settling dish" counts and air-centrifuge "volume counts" made in control atomisation experiments with Staph. albus/

Staph. albus incorporated in the suspension fluid. In experiments with small tubercle-bacillus-containing droplet-nuclei, averaging 2 to 4 microns in diameter, the number of tubercles developing in the rabbit's lungs (countable from 1 to 20,000) was approximately equal to the number of tubercle bacilli inhaled as indicated by concurrent sampling of the chamber air with an air-centrifuge. This finding suggests that almost 100% of small, 2 to 4 micron nuclei which are inhaled, reach and remain in the lung alveoli, and that a single tubercle bacillus planted on the alveolar lining is capable of inducing the development of a tuberculous lesion. In experiments made with large tubercle-bacillus-containing droplet-nuclei, averaging 12 to 15 microns in diameter, the number of tubercles developing in the rabbit's lungs was only 5 to 10% of the number of bacilli inhaled; presumably the majority of these large nuclei were filtered out of the air in the upper respiratory passages and ultimately swallowed; in the latter connection it was noted that none of the rabbits showed evidence of alimentary infection when sacrificed at 6 weeks. It was concluded that inhalation of a few tubercle bacilli in the (supposedly small) nuclei of droplets coughed or sneezed into the air, is of greater consequence than the inhalation of far larger numbers of bacilli in large (dust) particles which are strained out in the upper respiratory passages and swallowed.

Lurie/

Lurie and Abramson (1948) made further experiments by the technique of Wells and Lurie (1941). Of 29 rabbits of unknown genetic resistance, 1 rabbit inhaling 31 tubercle bacilli remained uninfected, while the other 28 rabbits inhaling from 32 to 10,116 bacilli all contracted pulmonary tuberculosis, the different animals developing from 1 to 1344 tubercles in rough but irregular proportion to the number of bacilli inhaled. On average, 1 lung tubercle was generated for every 9 bacilli inhaled; control experiments showed that nearly all the inhaled bacilli reached and remained in the lung alveoli. About 50 bacilli were inhaled by each of a further 5 rabbits which belonged to a genetically highly-resistant family and which had been vaccinated 6 months earlier; 1 rabbit remained uninfected, while the other 4 rabbits developed pulmonary tuberculosis with cavitation and without lymphogenous or haematogenous spread, that is a disease resembling the re-infection tuberculosis of human adults.

Glover (1944) exposed mice to a mist containing bovine-type tubercle bacilli; some mice were infected by inhalation of only 100 bacilli and almost all mice by 1000 bacilli. This high degree of susceptibility of mice to inhalation infection contrasts with their high resistance to infection by subcutaneous injection.

From all these observations just reviewed, it is concluded that by inhalation only very small numbers of/

of tubercle bacilli are required for regular induction of tuberculosis in the guinea-pig, mouse, rabbit, dog, goat and ox, a single bacillus sufficing in the guinea-pig and rabbit, while by ingestion very large numbers of bacilli, 1000 to 1,000,000 times the numbers effective by inhalation, are required for induction of tuberculosis in these animals, except in a minority of cases (e.g. about 10% of guinea-pigs in the series of Lange, 1924) when infection results from ingestion of only 100 or 1000 bacilli. It is not to be supposed that man will be an exception and that because of a higher resistance he will be unaffected by inhalation of only one or a few tubercle bacilli. As shown by Wells and Lurie (1941) and by Lurie and Abramson (1948) in rabbits, a high "resistance", acquired genetically or by vaccination, rarely conferred complete immunity even to very small numbers of bacilli (e.g. 20 to 100) acquired by inhalation, but usually manifested itself only in modifying the progress of the disease in the direction of chronicity and localisation.

In these comparative experiments the minimum infective doses by inhalation and by ingestion were determined for the induction of tuberculosis of any kind, not particularly of pulmonary tuberculosis. In fact, the animals infected by inhalation all contracted pulmonary tuberculosis, and the reports of many of the investigators make it clear that the lung infection/

infection was always primary, lesions occurring only in the lungs and bronchial glands at an early stage and the lung lesions appearing oldest at a later stage when generalised spread had occurred (Findel, 1907; Lange and Nowoselsky, 1925; Wells and Lurie, 1941; Wells, Ratcliffe and Crumb, 1948; Lurie and Abramson, 1948). In contrast, animals infected by ingestion contracted primary alimentary-tract tuberculosis; if they developed any lung lesions, these appeared at a late stage, secondary to lymphogenous or haematogenous spread; however, there have been some claims that primary pulmonary tuberculosis may be caused by ingestion of tubercle bacilli.

ii) Experimental Induction of Primary Pulmonary Tuberculosis by Feeding of Tubercle Bacilli.

Calmette and Guérin (1905) used an oesophageal tube to inoculate the stomachs of adult goats, giving 50 mg. of a bovine-type tubercle-bacillus culture on each of 4 days; one goat contracted localised ulcerative pulmonary tuberculosis without obvious involvement of the mesenteric glands, a primary tuberculosis of the lungs. On the other hand, when adult goats were fed naturally with enormous doses of tubercle bacilli (e.g. 5000 mg. per animal) given between slices of bread, they did not contract tuberculosis of any kind. Moreover, in young goats fed with 20 to 50 mg. of tubercle bacilli, either naturally or by oesophageal tube, the tuberculous lesions/

lesions appeared first in the mesenteric glands and in the lungs only at a later stage, in miliary form. It is difficult to understand why Calmette and Guérin should have considered these results as convincing evidence that pulmonary tuberculosis commonly is contracted by ingestion of tubercle bacilli.

Griffith (1907) fed 11 dogs with doses of 1 to 100 mg. of tubercle-bacillus culture; 3 dogs remained uninfected, 3 dogs developed tuberculosis both of the alimentary tract glands and of the lungs, and 5 dogs developed primary pulmonary tuberculosis with one or two small tubercles in the lungs and without tuberculous lesions in the alimentary canal and associated glands. In further experiments, Griffith found that 3 out of 14 monkeys fed with tubercle bacilli contracted pulmonary tuberculosis without coexistent abdominal lesions.

Cobbett (1917) pointed out that in such cases of pulmonary tuberculosis without mesenteric-gland disease, which is contracted after feeding of tubercle bacilli, the lung infection is by no means necessarily due to lymphatic spread from the intestines; the lung infection may be the result of spontaneous "animal-house cross-infection" acquired by inhalation, or the result of aspiration via the trachea from the mouth; when tubercle bacilli are introduced into the stomach by an oesophageal tube, the presence of the tube is likely/

likely to stimulate regurgitation and to facilitate aspiration through the glottis. Cobbett concludes that ingested tubercle bacilli will not, as a general rule, pass through the alimentary-tract lymph glands and cause disease in the lungs without causing disease in the glands and so marking the portal of entry and showing the lung infection not to be primary.

Lange (1924) found that in animals infected by mouth, the most extensive and thus primary lesions were in the cervical or mesenteric lymph glands; lung lesions occurred sometimes, but they appeared to be secondary to a miliary spread from the primary lesion elsewhere. In only 2 out of 30 guinea-pigs infected by mouth, was there any indication that the lung lesions possibly might be primary; these rare cases probably were due to aspiration of bacilli from mouth to lungs.

White and Minett (1941) made experiments with calves which provided support for Cobbett's view that primary pulmonary infections following natural feeding or feeding by an oesophageal tube are due not to lymphatic spread from the intestine but to aspiration down the trachea. When the bacilli were administered directly into the duodenum so as to exclude regurgitation and aspiration, the lungs never became infected.

iii)/

iii) Induction of Primary Lung Tuberculosis by Aspiration of Bacilli down Trachea from Mouth.

It may be concluded from these observations on animals that primary pulmonary tuberculosis, lung disease without evidence of previous disease of the cervical or mesenteric glands, probably never results from ingestion of bacilli followed by lymphatic spread from the tonsil or intestine, but sometimes may result from ingestion of bacilli followed by aspiration of the bacilli down the trachea. The findings of Cobbett (1917) on the passage of B. prodigiosus from the mouth via the trachea to the lungs of guinea-pigs, and the findings of Calmette and Guerin (1905), Griffith (1907), Lange (1924) and White and Minett (1941) on the occasional occurrence of primary pulmonary tuberculosis in animals following feeding of tubercle bacilli, suggest that aspiration from mouth via trachea to lungs can sometimes occur in such animals. The question arises as to whether such aspiration infection can occur at all readily in man; if it can occur, then primary pulmonary tuberculosis can result from the taking into the mouth of bacilli transmitted by contact, eating utensils or projectile droplets; if it can not occur, then it appears that contact and eating utensils are not of any importance in the transmission and causation of human pulmonary tuberculosis, since this is mainly a primary infection. There is not any direct evidence as to whether aspiration from mouth to lungs can occur readily in man;/

man; the question can be answered only from a consideration of the frequency of occurrence of human-type and bovine-type tubercle bacilli in primary pulmonary tuberculosis and primary alimentary-tract tuberculosis in man.

iv) Induction of Tuberculosis by Nasal and Conjunctival Inoculation.

Tuberculosis may be induced in animals by application of tubercle bacilli to the nasal mucosa or to the conjunctiva. The disease affects the site of inoculation and in the late stages shows lymphogenous and haematogenous spread. Sometimes, however, tubercle bacilli put into the nose may be aspirated down the trachea and cause primary tuberculosis of the lungs.

Cornet (quoted by Chapin, 1912) applied infective material by means of a feather to the nasal mucosa of guinea-pigs; he was able to produce disease of the nose and submaxillary glands.

Renshaw (1901) gently painted sputum on the nasal mucosa and in this way caused nasal tuberculosis in 7 out of 8 animals; spread to the lungs and other internal organs occurred after 6 weeks.

Lange (1924), working with guinea-pigs, infected the nose by placing in the anterior nasal cavity a drop of suspension containing a known mass of tubercle bacillus culture (100,000,000 living bacilli per mg.). With/

With doses of 0.000,000,1 mg. to 0.1 mg., 6 out of 36 guinea-pigs contracted tuberculosis; of those receiving 0.000,01 mg. or less, 2 out of 25 became tuberculous. Lange infected the conjunctiva by placing a drop of suspension into the sac and rubbing with the eyelids. With doses of 0.001 mg. to 0.000,000,5 mg., 4 out of 32 guinea-pigs contracted tuberculosis; of those receiving 0.000,01 mg. or less, 2 out of 6 became tuberculous.

Glover (1944) by intranasal instillation of about 50 bovine-type tubercle bacilli produced tuberculous lesions in the lungs of a proportion of mice so inoculated.

Men living in the environment of consumptives certainly collect tubercle bacilli in the nose, whether by inhalation of airborne bacilli or by introduction on the fingers in picking the nose. Straus (1894) by inoculation of animals found tubercle bacilli in the nasal secretion of 41% of 29 healthy persons, doctors, nurses and orderlies, who frequented wards and rooms occupied by tuberculous persons. Jones (1900) by inoculating animals with the nasal secretion of 31 persons not known to have come into close contact with consumptives, demonstrated the presence of tubercle bacilli in 3 cases. On the other hand, Le Noir and Camus (1908 a, b) could not demonstrate tubercle bacilli by inoculation of swabbings/

swabbings from the noses of the physicians and attendants of consumptives. Nasal tuberculosis in man is rare, and it does not seem likely on mechanical grounds that the few tubercle bacilli which could be collected in the anterior part of the nose during breathing, will commonly be aspirated into the lungs and so be an important cause of pulmonary tuberculosis.

v) "Animal-house Cross-infection".

The occurrence of tuberculosis cross-infections among experimental animals in the laboratory animal-house is of interest for two reasons; it necessitates great care in the organisation of tuberculosis induction experiments and caution in interpreting the results of these; and it illustrates the ready occurrence of airborne infection under natural conditions.

Lurie (1945) reviews the results of four of his investigations with guinea-pigs and rabbits. Normal guinea-pigs placed in individual cages in a room housing tuberculous animals acquired tuberculosis of respiratory origin, as evidenced by pulmonary lesions, massive tuberculous involvement of the draining tracheobronchial lymph glands and absence of disease in the cervical and mesenteric glands; infection by contact was precluded by wide separation of the cages. Similarly, normal rabbits contracted primary pulmonary tuberculosis when kept in separate cages in the same rooms/

rooms as rabbits excreting tubercle bacilli in the urine; these cross-infections among rabbits were prevented by ultraviolet irradiation of the room air, showing that the mechanism of infection was airborne.

(18) Anatomical Distribution of Primary-Infection Complex in Man.

If the infrequent anomalous cases of pulmonary tuberculosis in experimental animals developing after feeding with tubercle bacilli be attributed, as seems justifiable, to aspiration of the bacilli from the mouth down the trachea to the alveoli, then the observations made in infection experiments with animals establish clearly that the site of entry of the tubercle bacillus is always marked by the development there, and in the draining lymph-glands, of primary tuberculous lesions. The oldest or sole lesions are found in the lung and bronchial glands following infection by inhalation, and in the cervical or mesenteric glands following infection by ingestion.

On the basis of this distinction, evidence that pulmonary tuberculosis in man usually is acquired by inhalation (or aspiration) is provided by the usual finding at post-mortem examination of persons with latent or fatal pulmonary tuberculosis that the lungs exhibit the presence of primary-type lesions, focal or apical, while the intestines and mesenteric glands, and the tonsils and cervical glands, are unaffected or show only recent infection which is apparently secondary to the lung infection and due to swallowed sputum (Ghon, 1916; Opie, 1917 a, b, 1924 a, b, 1925; see also Cobbett, 1917). In some human pulmonary-tuberculosis necropsies there may be advanced tuberculous/

tuberculous lesions in the cervical or mesenteric glands, as well as in the lungs and bronchial glands, with nothing to show whether the alimentary-tract or respiratory-tract infection was earliest. In other necropsies the lung infection may appear obviously to be miliary and secondary to the alimentary infection. However, it may be stated that in the majority of cases the post-mortem appearances indicate clearly that the primary infection was in the lungs.

The relationship of tuberculosis of the tonsils, adenoids and cervical lymph glands to pulmonary tuberculosis is of interest. It appears that tuberculosis of the cervical lymph-glands is usually secondary to tuberculosis of the tonsil, which acts as the site of entry for tubercle bacilli from the mouth and which drains to the jugulo-digastric and upper deep cervical lymph-glands. Webster (1932) found tuberculous lesions, mostly of microscopical dimensions, in the tonsils of 40 out of 86 children with tuberculous cervical adenitis who submitted to tonsillectomy. In other cases tuberculosis of the cervical glands may be secondary to tuberculosis of the nasopharyngeal tonsil, the "adenoids", which receives inhaled tubercle bacilli from the nose or tubercle bacilli from sputum coughed from lung to throat, and which drains to the retropharyngeal and upper deep cervical lymph-glands. Tuberculosis of hyperplastic "adenoids" is by no means uncommon, occurring/

occurring in about 10% of such cases (see Lartigau and Nicoll, 1902). Rossner (1933) distinguished between primary or exogenous infection and secondary or endogenous infection of the tonsil; in the former case the tubercle bacilli are acquired by ingestion or inhalation and in the latter case by sputum from the lungs or, possibly, by lymphogenous or haematogenous spread from the lungs or other infected organs. It seems probable that a large proportion of tuberculous infections of the tonsils and cervical glands are exogenous, since many cervical-gland infections occur in otherwise healthy persons and since about 50% of the gland infections are due to the bovine-type of tubercle bacillus which presumably is acquired by ingestion in milk with direct passage from the mouth into the tonsil, and could not commonly be acquired by spread from lung lesions which are almost always due to the human-type bacillus. Secondary tonsillar- and cervical-gland tuberculosis does, however, occur commonly among patients with pulmonary tuberculosis, presumably from sputum passing over the tonsils; Walsham (1903) found tuberculous lesions in the cervical glands of a large proportion of 27 fatal cases of pulmonary tuberculosis; Vlasto (1931) quoted Strassmann as having found at post-mortem examination tuberculous lesions in the tonsils of 13 out of 21 patients.

## (19) Relative Frequency of Occurrence of Human-type and Bovine-type Tubercle Bacilli in Pulmonary Tuberculosis and Alimentary-Tract Tuberculosis.

Typing of the tubercle bacilli isolated from the sputum of patients with pulmonary tuberculosis has made it abundantly clear that the great majority of such pulmonary infections are due to the human-type bacillus. Griffith (1914) reviewed 938 cases of pulmonary tuberculosis examined by 26 investigators in different countries; only 3 of the 938 cases (0.32%) were due to infection with a bovine-type bacillus alone, while another 3 cases were mixed human-type and bovine-type infections. Lange (1932) reported on 345 German cases of tuberculosis of the lungs and bronchial glands; only 2 of the 345 cases (0.6%) were due to a bovine-type bacillus alone, and a further 2 cases were mixed infections; the 283 of the 345 cases which were adults, included the two mixed infections but neither of the pure bovine-type infections. Griffith and Munro (1943) summarised investigations of 6963 cases of pulmonary tuberculosis in Great Britain; only 241 of the 6963 cases (3.5%) were due to the bovine-type bacillus; the percentage of bovine-type infections was highest in the rural north and lowest in the south, being 25.8% in the Orkney Islands, 9.1% in rural north-east Scotland, 5.2% in the rest of Scotland, 2% in north and middle England, 0.6% in south England, 1% in Wales and 0% in Eire (the average for 2769 cases in Scotland was 5.8%, and for 3671 cases in England

was/

was 2.2%). Cutbill and Lynn (1944) investigated 2101 cases of pulmonary tuberculosis in a sanatorium in England, and found that only 48 cases (2.3%) were due to the bovine-type bacillus. It may be concluded that the bovine-type tubercle bacillus is responsible for only about 2% of cases of pulmonary tuberculosis.

In marked contrast to this, the bovine-type bacillus is responsible for a large proportion of cases of alimentary-tract tuberculosis. Griffith (1938) reported 50% of 128 English cases of tuberculous cervical lymphadenitis as being due to the bovine-type bacillus. Cobbett (1917) summarising investigations made in Scotland, England, Germany and the U.S.A., reported that the bovine-type bacillus was the infecting organism in 140 out of 249 cases (56%) of tuberculous cervical lymphadenitis, occurring in 9 out of 49 adult cases (18.3%) and in 131 out of 200 cases (66%) in children under 16 years of age, and also was the infecting organism in 28 out of 55 cases (51%) of primary abdominal tuberculosis (mainly mesenteric lymphadenitis) occurring in none of 8 adult cases and in 28 of 47 cases (60%) in children under 16 years. Blacklock (1932) examining 101 cases of primary abdominal tuberculosis in children, isolated 12 human-type strains and 54 bovine-type strains; about 80% of the cases were due to the bovine-type bacillus. It may be concluded that the bovine-type tubercle bacillus is responsible for at least 50% of cases/

cases of alimentary-tract tuberculosis (i.e. of cervical and of mesenteric glands).

It may be assumed that primary alimentary-tract tuberculosis is acquired by ingestion of tubercle bacilli taken into the mouth either in infected milk, as in the case of the bovine-type bacillus, or on vehicles of contact infection such as the fingers and eating utensils, as in the case of the human-type bacillus. The proportions of cases of primary alimentary-tract tuberculosis due respectively to the bovine-type bacillus and the human-type bacillus, approximately 50% and 50%, may fairly be taken to indicate the normal extent to which persons are exposed to ingestion of the two kinds of bacillus. In fact, bovine-type bacilli must as often enter persons' mouths as human-type bacilli. Therefore, if it were readily possible for bacilli acquired by ingestion into the mouth to give rise to pulmonary tuberculosis, whether by aspiration via the trachea to the lung or by absorption from intestine via lymphatics to the lung, pulmonary tuberculosis should surely be due to the bovine-type bacillus as commonly as is alimentary-tract tuberculosis, namely in about 50% of cases. But, as has been shown, pulmonary tuberculosis is due to the bovine-type bacillus only in about 2% of cases. This disproportionate infrequency of bovine-type infections of the lungs seems/

seems explicable only if it is very difficult for ingestion of bacilli to give rise to pulmonary tuberculosis. Thus, it may be concluded that pulmonary tuberculosis rarely is caused by tubercle bacilli received into the mouth and therefore rarely is transmitted by contact or eating utensils. Pulmonary tuberculosis must usually be contracted by inhalation of airborne infected dust-particles or droplet-nuclei.

(20) Mode of Infection in Pulmonary Tuberculosis due to Bovine-type Tubercle Bacilli.

Not a great deal of information is available as to the mode of infection in the infrequent cases of pulmonary tuberculosis which are due to the bovine-type tubercle bacillus.

Lange (1932) advanced evidence that primary pulmonary tuberculosis due to the bovine-type bacillus might be acquired directly by contact with tuberculous cows, for instance by inhaling infected dust produced by the cows. He reported that out of 40 cases of pulmonary tuberculosis in adults who were probably exposed to tuberculous cows by their occupation (e.g. milkers), 8 cases (20%) were pure bovine infections; in contrast, out of 283 other cases of pulmonary tuberculosis in adults (who were not so exposed), none showed pure infection with bovine-type bacilli and only 2 showed mixed infections.

Griffith and Munro (1943) concluded that the majority of their 241 bovine-type cases of pulmonary tuberculosis were due to ingestion of infected cow's milk followed by secondary spread from alimentary tract to lung; a quarter to a third of their cases showed clear clinical indication of previous alimentary tuberculosis (e.g. cervical adenitis or calcareous mesenteric glands), while many of the other had consumed large quantities of raw cow's milk in childhood; out of 14 cases examined post-mortem,

9 showed anatomical evidence that the alimentary canal was the route of infection.

Among their 48 bovine-type cases of pulmonary tuberculosis, Cutbill and Lynn (1944) found evidence in 16 cases (33%) that infection probably took place via the alimentary canal by ingestion of infected milk (history of drinking raw milk and clinical evidence of previous alimentary-tract infection), and in 10 cases (21%) that infection probably took place by "contact" with infected cattle (history of not drinking raw milk, history of contact with cattle as in farm-work, and absence of clinical evidence of previous alimentary-tract infection), while in 19 cases they did not find any conclusive evidence as to the mode of infection. Infection by "contact" (i.e. in the family) with another human case of bovine-type pulmonary tuberculosis was found responsible for only 4 out of 241 bovine-type cases studied by Griffith and Munro, and for only 3 out of 48 bovine-type cases studied by Cutbill and Lynn.

(21) Relative Importance of Dust and Droplet-Nuclei as Agents of Airborne Infection.

While it is now generally accepted that pulmonary tuberculosis is acquired by inhalation of airborne tubercle bacilli, and not by ingestion, there still is a sharp division of opinion as to whether the airborne infection is due mainly to dust, as argued by Koch, Cornet, Chaussé and Lange, or mainly to "droplets", as argued by Flügge and Wells.

Wells, Winslow and Robertson (1946) state that droplet-nuclei are the main vehicle of airborne infections of all kinds and in particular of lung infections such as pulmonary tuberculosis. They draw a sharp distinction between dustborne air infection and droplet-nucleus air infection according to particle size and duration of air carriage; infected droplet-nuclei are small (2 to 10 microns), remain airborne for long periods, are dispersed widely indoors and can penetrate to the lungs; infected dust-particles are large (10 to 100 microns), settle rapidly giving only localised and brief air contamination, and cause infections of the nose and throat only. Again, Wells, Ratcliffe and Crumb (1948) state that the infected droplet-nuclei of cough-spray are especially important in the causation of pulmonary tuberculosis because their very small size allows them to escape removal in the upper respiratory passages and to pass freely into the lungs./

lungs.

It must be stated that Wells and his colleagues entirely fail to substantiate this distinction which they make. They do not explain their reasons for stating that the infected droplet-nuclei of cough-spray are very small, 2 to 10 microns, and that infected dust-particles are large, 10 to 100 microns. Apparently, they assume that a consumptive's coughing will produce infected droplet-nuclei as readily and of as small a size (under 5 microns) as the culture-suspension atomiser used in their apparatus for experimental infection of animals; such an assumption is quite unjustifiable and, in fact, direct observations on the cough-spray of consumptives by Heymann (1901), Chaussé (1914 a, b; 1916) and Hippke (1921), which apparently have been ignored by Wells and his colleagues, show that small infected droplet-nuclei are only rarely and scantily produced. The average settling-velocity figures given by Wells, Winslow and Robertson (1946) for the bacteria-carrying dust-particles of the air of various environments, vary mainly from ½ to 3 feet per minute, corresponding to average particle diameters of 10 to 25 microns. These authors apparently disregard the great size-heterogeneity of dust; if 20 microns is the average diameter of the infected dust-particles, probably there will be numerous infected dust-particles smaller than 5 microns. Furthermore/

Furthermore, it is not justifiable to draw conclusions about the size of the infected dust-particles which will be given off by sputum-soiled materials, from measurements of the bacteria-carrying dust-particles normally found in the air, that is particles carrying bacteria of all kinds other than tubercle bacilli. The only valid way of determining the relative importance of dust and droplet-nuclei in causing pulmonary tuberculosis is by direct comparative observations of the infected dust-particles and the infected droplet-nuclei which are produced naturally from consumptives and their environment. Thus, definite conclusions can not be drawn from the findings of Köhlisch (1908) and Lange and Keschischian (1925) as to the proportion of inhaled particles which reach the lungs, from artificial dusts and sprays (2 to 12% for "dusts", and 20 to 30% for "droplets").

The investigations which are helpful in elucidating the relative importance of dust and droplet-nuclei, are those of Laschtschenko (1899), Heymann (1899, 1901), Chaussé (1914 a, b; 1916) and Hippke (1921) on the production by consumptives' coughing of airborne infection with "droplets" (droplet-nuclei) containing tubercle bacilli, and those of Sticher (1899), Heymann (1901), Chaussé (1913 c, d; 1914 c) and Lange (1926) on the production of airborne infection with dust-particles carrying/

carrying tubercle bacilli, by the brushing and shaking of cloths and handkerchiefs which were soiled naturally or were smeared or sprayed with sputum artificially. After careful consideration and comparison of the results of these investigations, I conclude that airborne infection is more likely to be due to dust than to droplet-nuclei, and that dust-raising from handkerchiefs, clothes and bedding is a much more important cause of pulmonary tuberculosis than is the droplet-spray of a consumptive's coughing.

Heymann (1901) demonstrated fairly frequently the production of tubercle-bacillus-carrying dust-particles which remained airborne for as much as 15, 30 or 60 minutes, and only rarely (and doubtfully) the production of tubercle-bacillus-containing "droplets" (nuclei) which remained airborne for as much as 30 minutes; the dustborne air infection was the more readily produced and its long persistence proved that the infected dust particles were very small, not more than a few microns in diameter, and thus capable of being inhaled directly to the lung alveoli. In the experiments of Chaussé (1913 c, d; 1914 a, b; 1916), pulmonary tuberculosis was contracted by the great majority of guinea-pigs exposed in a chamber to air contaminated with dust brushed or shaken from soiled handkerchiefs or woollen cloths, but by only a minority of guinea-pigs exposed to the concentrated spray of numerous coughs by patients with open pulmonary tuberculosis.

(22) Conclusions.

Pulmonary tuberculosis is contracted mainly by inhalation into the lungs of small airborne infected dust-particles liberated from handkerchiefs, clothes, bedding and carpets which have been soiled by the sputum or by the large projectile cough-droplets of patients.

A much less important, but possibly not rare, mode of infection is by inhalation of small infected droplet-nuclei introduced into the air by the coughing of patients.

A third, and probably rare, mode of infection is by ingestion into the mouth, on fingers or eating utensils, of tubercle bacilli acquired from patients by contact, with subsequent aspiration of the bacilli, probably in small droplets of saliva, from mouth to lungs via the trachea.

Tubercle bacilli acquired by ingestion, and invading the body through the tonsils or intestines, do not give rise to pulmonary tuberculosis apart from miliary lung infection due to haematogenous spread from a primary alimentary-tract lesion.

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