A STUDY OF THE BACTERIOLOGY AND PATHOLOGY OF TUBERCULOUS DISEASE IN INFANCY AND CHILDHOOD

by

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INTRODUCTION.

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The present investigation appeared to be warranted in view of the great difference, found by various workers, in the incidence of bovine infections in children in England and the continent as compared with Scotland (chiefly Edinburgh). Up to the present no large series of figures has been published for Glasgow and the West of Scotland. In the city itself most of the milk is drawn from the Ayrshire herds which are under very strict veterinary inspection, and, according to MacGregor (1930), 80 to 90 per cent. of this is pasteurised before distribution, so that Wright (1929), comparing the returns for the milk supplies of three different Scottish cities, found that 2.6 per cent. of the Glasgow samples tested were infected with tubercle as compared with 8.5 per cent. for Edinburgh, and 5.5 for Aberdeen. It is to be noted, however, that the method employed for testing samples in each area was different. Factors such as this might materially affect the incidence of bovine infections in children in the Glasgow area as compared with Edinburgh. The chances of bovine infection from milk, however, would appear not to be great in most parts of Great Britain. According to/

to Savage (1929) veterinary data indicated that about 40 per cent. of cows were affected with tuberculosis, the great majority of these being non-infective to other animals, and, while there were no reliable statistics available as to the percentage of "open" cases, he was of the opinion that probably less than one per cent. of cows developed "open" tuberculosis. Further, with the mixing of milk obtained from various herds, the numbers of tubercle bacilli will be still further reduced. There is little doubt that small numbers of viable bovine tubercle bacilli may be swallowed by the healthy child without any recognisable lesion resulting, as in the cases cited by Holt (1922) in America and by Koch (1929) in Germany. Lichtenstein (1923), on investigating the death-rate due to tuberculosis during the first 5 years of life and comparing this with the incidence of tuberculosis in cattle in the different counties in Sweden, found that there was no noteworthy connection between the two. On the contrary, there was a close relationship between the number of cases of tuberculosis in children and the frequency of tuberculosis among the population as a whole.

Further, an opportunity was afforded of investigating a large series of cases of tuberculosis in infancy and childhood, and ascertaining to what extent death could be attributed to the various types of tubercle bacilli, and/

and with what particular pathological lesions the various strains were associated. Also, it might be possible with the combined bacteriological and pathological findings to give a more definite opinion regarding the portal of entry of the infection than if only one side of the subject was investigated.

The children who formed the subjects of this investigation were mostly those of poor or working-class people, and the majority dwelt in Glasgow or the surrounding districts. Where the locus of habitation was of particular importance this fact has been mentioned. Apart from cases occurring while we were absent, they were consecutive, and, accordingly, as far as we were concerned, were not selected. On the clinical side, however, there was a certain amount of uncontrollable selection in favour of non-tuberculous cases. Further, there was the possibility of selection outside the hospital, many of the more chronic cases being sent by their own doctors to special tuberculosis cliniques and hospitals. Such selection must be expected in these days when special hospitals and cliniques are available for the treatment exclusively of tuberculosis. Accordingly, we are of the opinion that any strict comparison cannot be made of the number of cases showing tuberculous lesions with similar statistics from other hospitals. This more particularly applies to reports/

reports of investigations on this subject published in the past when special facilities were not available for the treatment of tuberculosis to such an extent as at the present time. A comparison, however, may be possible to some extent with infants and children up to 3 years of age in whom more acute lesions were present, e.g., meningitis. In many of these young children the pulmonary or abdominal disease is not readily diagnosed unless by the aid of radiology in the one case, or by deep abdominal palpation under general anaesthesia in the other. The real nature of the disease causing the cerebral symptoms is thus often recognised only in hospital. Further, the figures given here cannot be regarded as indicating the incidence of tuberculosis in the general child population. It must be remembered that we were dealing in the great majority of cases with sick children in some of whom the fatal illness may have activated small foci of tuberculosis, which, if the child had remained healthy, would probably have retrogressed. In this connection also there has to be considered the question of tubercle bacilli lying latent in the tonsils or lymphatic glands which appear normal to the naked-eye. Such cases were described originally by Loomis (1890) and Pizzini (1892) in the case of the bronchial glands, and later for other groups of lymph nodes as well as the bronchial by Macfadyen and McConkey/

McConkey (1903), Eastwood, A. S. and F. Griffith (1914) in this country, by Harbitz (1905) and de Besche (1913) in Norway, and by Kälble (1899), Weichselbaum and Bartel (1905), Gaffky (1907), Rothe (1911), and Ungermann (1912) in Germany. Some of these workers actually halved the glands, and in some cases the part used for inoculation produced tuberculosis in the guinea-pig, while the other half on microscopic examination showed no histological change. It is possible, however, that in some of the cases reported by these workers a small tuberculous lesion may have been missed though present at the site of entry of the infection in the territory which the lymph nodes drained. Apart from this, these observations are of importance as they show that it is possible for the bacilli, after being carried in phagocytic cells through a mucous surface, to be retained in apparently normal lymphatic glands in relation to the portal of entry, or passed through successive groups without causing any lesion. If the organisms were present in healthy glands at a distance from the portal of entry, and through some local inflammatory disease the resistance of the glands was lowered, then the latent bacilli which might have been destroyed had the gland remained healthy might now be able to produce definite lesions.

The natural resistance of the tissues to tuberculous infection, and the fact that it can be lowered by diseases accompanied/

accompanied by toxaemia, are important factors in the pathology of tuberculosis in children. As an example of this, the simple case of tuberculous cervical glands in children who have their heads infested with lice can be cited. Here a localised toxic effect is at work, and as a result of slight, often unrecognisable, septic infection of the lice bites the cervical, occipital and lymphatic glands become the seat of sub-acute lymphadenitis. Such is noted clinically by a very moderate generalised enlargement and tenderness of these glands. In some of these cases a tuberculous infection is frequently superimposed. Further, the catarrhal ailments in children associated with the respiratory tract may stir up a quiescent focus of tuberculosis in either the lungs or bronchial glands. As will be afterwards pointed out, fatal tuberculosis associated with the respiratory system was found in this investigation to be commoner in the first half of the year as compared with the second; that is, at the period immediately after that during which children suffer most from minor and major respiratory diseases when the normal resistance of the tissues of the respiratory tract would be lowered.

Material.

In this investigation material from two sources has been available for study:-

(1) Autopsy Cases.

A combined pathological and bacteriological study was made of definite and suspicious tuberculous lesions occurring in a series of 1,300 consecutive post-mortems at the Royal Hospital for Sick Children, Glasgow, and extending over a period of six years (March, 1924, to February, 1930). At the autopsies the whole of the respiratory system, larynx, trachea, bronchi and lungs, as well as the various groups of thoracic lymph nodes, were investigated for any evidence of tuberculosis. Similarly, the gastro-intestinal tract from the posterior part of the pharynx to the rectum, and the various abdominal lymphatic glands and the other abdominal viscera were also searched minutely. In the case of the pharynx and mouth, however, it was not always possible to conduct an extensive investigation. Those giving permission for autopsies on children have occasionally particularly requested that such should be as limited as possible, so that in a few cases it was considered undesirable to perform any extensive dissection of the neck. In all of the subjects, however, the various structures in the lower part of the neck up to the level of the larynx were searched for evidence of any tuberculous lesion. The brain, meninges and middle ears were also/

also examined. In a few cases permission to examine the head and its contents was not given. In these the presence of a cerebral lesion was diagnosed from the clinical symptoms as well as from the bacteriological and cytological findings in the cerebro-spinal fluid drawn off during life.

The autopsies were nearly all performed by myself, a few being done by my assistants under my personal supervision. Accordingly, the criteria as to what was considered a tuberculous lesion were constant throughout the whole series. For a diagnosis of tuberculosis, we adopted the criteria that the lesions in the organs, lymphatic glands or tissues should show evidence of nodular tubercle formation, caseation, or the other retrogressive changes associated with tuberculous lesions. When such were present in any of the organs, the associated lymphatic glands were also carefully examined, and the degree and path of the extension of the morbid process noted. In the case of a suspected tuberculous exudate in the pleural or peritoneal sacs or in joints, evidence of tuberculous lesions was looked for in the surrounding tissues. Where any lesion was suspected of having a tuberculous origin, e.g., scars in the lungs, isolated pleural adhesions, doubtful foci in lymphatic glands, such were submitted to histological examination before a diagnosis was made.
In/

In this, however, no attempt was made to cut serial sections, a few sections only being made from the parts which were actually under suspicion.

The number of cases with tuberculous lesions recognisable by the naked-eye or where doubtful by microscopic examination in this series was 216 (16.6 per cent. of 1,300 autopsies). In all of them the situation of the most advanced lesion either in the organs themselves or in the related lymph nodes was, as far as possible, decided. This would provide a useful basis for classifying our results, and also might help in deciding the probable portal of entry of the infection, though this point is discussed more fully later. There were 3 cases in which it was evident from the lesions noted at post-mortem that there was a double portal of infection, thus making a total of 219 lesions which were regarded as probable primary sites of infection, and from which an attempt was made to isolate the infecting organisms. The bacteriological work associated with the lesions was, of a necessity, performed first, and in doing this much material was sacrificed at the expense of any subsequent histological investigation that might be necessary. It was considered desirable, particularly in the case of lesions showing extreme caseation, especially lymphatic glands, to take fairly/

fairly large masses of tissue so as to include the surrounding area of reaction. By doing this we avoided any risk of losing the infecting virus by using material which was chiefly caseous and which in many cases, though showing stainable bacilli, has been found by numerous workers, as reviewed by Griffith (1929), to yield negative results on culture and inoculation into guinea-pigs. In 36 specimens failure to isolate the virus resulted. From the remainder, 183 strains were obtained, while in 6 cases bacilli were also isolated from secondary lesions as well, making a total of 189 strains obtained from autopsy material. No attempt was made in this investigation to find the frequency of latent tuberculous infection in normal tissues as it was decided that no useful purpose would be served by doing so in an unselected series of cases since it would be impossible to say whether or not such infection was recent, and the bacilli in process of destruction. We have used the word <u>latent</u> only in reference to those cases where the tubercle bacilli were present in tissues or organs which showed no recognisable morbid change. On the other hand, other authors have used the same term in reference to definite tuberculous lesions which were not the cause of death, and which, though not recognised during life, were found at autopsy.

(2) Surgical Cases.

The other source of tuberculous lesion consisted of 65 specimens sent for examination from the surgical wards. The ages of children in this group varied from 7 months to 12 years, 11 (16.9 per cent.) being under 2 years. The paucity of this material was due to the fact that, as far as possible, cases of this nature were treated at special tuberculosis cliniques outside the hospital. From this material 52 strains were isolated.

SECTION I.

Methods used in the isolation of the infecting virus.

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(1) Preparation of the inoculum.

The two usual methods of isolating the infecting virus are firstly by inoculation of guinea-pigs with emulsions of the tuberculous tissue or pathological exudates and then making cultures from the lesions produced in the animal, or secondly by direct culture of the virus. The former method has been most extensively employed, but to be a success at least two guinea-pigs should be inoculated from each specimen, as in the event of one dying of intercurrent disease before the inoculated bacilli have had time to increase, the investigator has still the other animal to fall back on. Besides being somewhat costly, more animal space is required, and even with the best of care guinea-pigs often die soon after inoculation for no apparent reason, while at other times an epidemic of enteritis will occur in the animal house and kill most of the stock. Along with these disadvantages there is to be considered the very occasional failure, as was experienced in this investigation, to produce a lesion in guinea-pigs with small numbers of tubercle bacilli. A further drawback to the sole use of guinea-pigs for isolation is the rare occurrence in children and young adults of/

of an avian type of infection to which the guinea-pig is resistant when inoculated subcutaneously with small doses. Such types of infection occurring in man have been reviewed by Gasul (1929), and while they are uncommon, yet the fact is worth bearing in mind when undertaking an investigation of tuberculous disease in children, though the clinical or post-mortem findings would point to some unusual type of tuberculous disease. In the present series, however, no examples of avian tuberculosis were noted. The cultural method has also serious drawbacks, chiefly on account of the transference of a strictly parasitic organism like the tubercle bacillus, often in small numbers, from its natural habitat in animal tissues to the artificial conditions of even the most satisfactory culture medium requiring, in most instances, time for the adaptation of the organism to its new source of nourishment, and before the virus has had time for this to occur it may perish, and accordingly a strain is lost. In this investigation it was found that some strains of the tubercle bacillus were more rapidly adaptable to culture media than others. By some workers direct inoculation of the infected specimen into the subcutaneous tissues of the rabbit has been used as a means of typing the infecting virus, but obviously such a procedure is open to serious criticism. For example, though tubercle bacilli may be seen in stained/

stained films, yet this is not sufficient proof that such are viable. If no lesion developed from an inoculation where the bacilli were dead and yet stainable, the worker would conclude that the virus was of low virulence for the rabbit, and consequently of human type. Further, the various methods of ascertaining the numbers of the bacilli in such an inoculum are tedious procedures which are often neglected, so that any comparison of the lesions produced in different animals with the dose inoculated is not possible.

Isolation by means of a combination of direct culture and guinea-pig experiments suggested itself as being useful in the present investigation and suited to the conditions under which it was conducted, as if the former, which was used in all cases, was successful, much time would be saved, while guinea-pigs were reserved, as is described more fully later, for selected cases where there was likely to be some difficulty in isolating the infecting virus by means of culture alone. In nearly all the cases, and particularly in the autopsy ones as already noted, fairly large portions of diseased tissue or infected glands were used for isolation of the infecting virus. This ensured the inclusion of areas where the disease was progressive and likely to contain living bacilli. Where possible only half of the specimen was used for isolation, the/

the remainder being put in a sterile bottle and kept in the refrigerator till the results of isolation experiments were available.

As most of the tissue was obtained post-mortem, it was necessary to adopt some means of destroying contaminating organisms. This was particularly the case with specimens obtained from lung, tracheo-bronchial or mesenteric glands, which in children dying from tuberculosis nearly always contained other organisms, due largely to the occurrence of a terminal simple acute pneumonia in the one case or a slight acute enteritis in the other. Some preliminary experiments were however tried with material taken as cleanly as possible from the lesions found at autopsy and ground up in saline before being injected subcutaneously into guinea-pigs. Out of the 9 animals so inoculated 2 died a few days after inoculation, one with acute cellulitis in the region surrounding the site of inoculation, and the other with a spreading gaseous gangrenous lesion due to an organism of the B. Welchii type. Eastwood and F. Griffith (1914 (1)) experienced similar difficulty while investigating tuberculous lesions in children, and to overcome this treated some of their specimens before inoculation with a low concentration of antiformin. It was thus obvious that, unless a fair number of opportunities of isolating the infecting virus were to be/

be lost, one or other of the reagents used to destroy organisms other than the tubercle bacillus must be used. On surveying the various procedures adopted for this, the antiformin method seemed to be the most suitable and was the one with which previous experience had been obtained.

The concentration of antiformin and the duration of treatment adopted by different workers on the subject have varied much. Griffith (1914 (1 & 2), working with sputa and tissues, used an ultimate concentration of 5 per cent. for 5 to 30 minutes, though later (1916 (1)) he recommended 15 per cent. for sputa and tissues and made cultures from the mixture at intervals starting after 2 minutes' exposure and ending at 20 minutes. The Royal Commission (1911 (1)) found that 10 per cent. for 10 minutes yielded pure growths from sputa, while in the case of thick sputa the tubercle bacilli were still viable, though diminished in numbers, after 24 hours' exposure. Cruickshank (1912) used a concentration of 15 per cent. for sputum for 1 to 2 hours and was able to isolate the infecting virus in 60 out of 70 specimens examined. Brown and Smith (1910) used a similar concentration for one hour at room temperature and were able to isolate the infecting virus from sputa in 97 per cent. of specimens where films made from the antiformin sediment showed/

showed tubercle bacilli on microscopic examination and from 27 per cent. where no bacilli were seen in films of the sediment. Paterson (1910) worked with a concentration of 20 per cent. for 4 to 6 hours at 37°C., or 24 hours at room temperature, but did not appear to have succeeded well with isolation by culture, ascribing his failure as probably due to the use of a too concentrated solution of antiformin. Uhlenhuth and Xylander (1908), who brought the antiformin method into prominence, demonstrated that tubercle bacilli, after treatment with 20 per cent. antiformin for 24 hours, were still pathogenic to animals, while Weber and Dieterlen (1912) made the important observation that bacilli isolated by this means suffered no loss of virulence when compared with those isolated by direct inoculation of guinea-pigs from the same source.

As the material with which we were concerned was mostly tissue, it was decided that a concentration of 7.5 per cent. antiformin for a period of 20 to 30 minutes would be suitable. Though the concentration was a little higher than that used by some workers and the time longer, yet it was probable that the tissues would protect the tubercle bacilli from the action of the antiformin better than the sputa; and, further, a finer emulsion of tissue would be obtained. There is no doubt, however, that even such a low concentration destroyed a few strains of tubercle/

tubercle bacilli. With others the number of viable bacilli was materially reduced. In such cases numerous tubercle bacilli were seen in films made from antiformin sediments prepared from acute lesions, yet no growth resulted on culture and the inoculated guinea-pigs showed no naked-eye or microscopic evidence of tubercle. In some cases where only cultures were made these remained sterile or showed very few colonies. The most striking example of this was in one guinea-pig inoculated sub-cutaneously with a bovine strain. At post-mortem tuberculous lesions were found in many of the lymphatic glands, and in smears made from these the bacilli were particularly abundant. The inguinal lymph nodes were removed aseptically and cultures were sown directly with material from them. The same mass of glands was then treated with antiformin as described later, and further cultures made from the washed sediment. In the former cultures a rich growth resulted, while in the latter there were only a few colonies. The explanation of this irregular deleterious effect of antiformin on the vitality of the tubercle bacillus is difficult to explain. It may be that certain strains are more susceptible than others, as shown by Donges (1913) who, working with tuberculous sputa and discharges from tuberculous cattle, found on comparing one human and two bovine strains that one of the/

the latter was less resistant to high concentrations of antiformin than the other strains, as tested by the results produced on inoculation of guinea-pigs. In some cases the tissue or the pathological exudate in which the tubercle bacillus is present may not protect the organism as well as in others. This has been demonstrated by Uhlenhuth and Xylander (1909) who found that a suspension in water of bacilli from a culture was killed by 20 per cent. antiformin in from 6 to 12 hours; whereas in the case of tuberculous sputa treated with concentrated antiformin for 6 hours the tubercle bacilli were still alive and capable of infecting animals. This they attributed to the albuminous substances in the sputa protecting the bacilli. Further, under certain conditions the tubercle bacillus does not develop to the full its waxy envelope, such bacilli being stainable by the ordinary Gram's method and appearing as a blue, rather than the usual black, fine diphtheroid bacillus. This observation was first made by Browning and Gulbransen in mice experimentally infected with tubercle bacilli, and has also been noted many times by us in the present investigation in caseous glands and in the meninges. With these facts in view, it is not possible to state with certainty in what proportion of cases the infecting bacilli were dead where cultural and guinea-pig tests gave negative results/

results after being inoculated with washed antiformin sediments.

As guinea-pigs were also largely used for isolation of the infecting virus in addition to cultures, to make conditions comparable it was necessary to use the same sediment for each. It was undesirable, however, to inoculate the tissue-antiformin mixture directly into the subcutaneous tissues of the guinea-pig, as when such was tried on a few occasions an acute chemical oedematous inflammation resulted, with, in one case, sloughing and consequent ulceration. The extent and severity of the inflammatory lesion in these cases depended largely on the amount of the inoculum. Accordingly, all sediments obtained on centrifuging the tissue-antiformin mixture were washed twice with distilled water, a process which could have been omitted if it had been possible to know beforehand that only cultures would be necessary for isolation in the case under investigation as the amount of unreduced antiformin remaining in the sediment was not sufficient to interfere with growth.

The material from the lesions found at autopsy was removed as cleanly as possible, was cut into very small pieces with forceps and scissors in a sterile mortar, and then thoroughly ground with the pestal. An equal part of a 15 per cent. solution of antiformin in distilled/

distilled water was then added and the grinding process repeated, after which the mortar was covered and placed in the incubator for 10 minutes. At the end of this time the mixture was well ground up again, transferred to a thick-walled tube, and centrifuged at high speed (3,000 revolutions) for 10 minutes, when the supernatant fluid was pipetted off and the sediment mixed with 20 c.c. distilled water and again rapidly centrifuged, after which the supernatant fluid was pipetted off. This washing was repeated once. We always drew off the supernatant fluid with a pipette as this did not disturb the top portion of the sediment in which the tubercle bacilli were often numerous. By pouring off the supernatant fluid a portion of the top of the sediment is often lost as well. The instruments, glass-ware, and water used in these manipulations were all sterilised beforehand. With the above method, complete solution of the material under investigation did not generally occur, with the exception of soft tissues such as caseous glands, but this did not seem a disadvantage. Sterile distilled water was found to be better than saline both for diluting the antiformin and also for the subsequent washings of the sediment, parallel experiments with distilled water and saline showing a greater amount of growth with several strains, particularly those of bovine type, when the former was used. All/

All specimens, with few exceptions, were treated immediately on being taken from the autopsy, or on receipt from the surgical theatres, as it was observed that if the tissue was kept in the ice-box for a few days before being treated failure to isolate the organisms often occurred. In this connection the observations of Richet (1927) are of interest. This worker found that on mixing a thin emulsion of a human strain of tubercle bacilli and fresh tissue (liver, spleen and muscle of rabbit, and liver of guinea-pig) and leaving for 12 to 14 days the mixture produced no lesion when injected into guinea-pigs. This he attributed to the lytic action of the tissues on the tubercle bacillus and found that the principle responsible was thermolabile. Films were made from each washed sediment, stained by Ziehl-Neelsen's method, and examined with an oil immersion objective to ascertain the bacterial content. A sediment or emulsion was classified as being <u>abundant</u> (+++) when many tubercle bacilli were found in every field, as <u>numerous</u> (++) when bacilli occurred in every second or third field, as <u>scanty</u> (+) when the organisms were less numerous, and as <u>negative</u> (-) when no bacilli could be found after ten minutes' search. Providing the numbers of tubercle bacilli fell into the first two categories and no sporing organisms were present, cultures only were put up; otherwise/

otherwise, i.e., when the tubercle bacilli were scanty, absent, or organisms likely to give rise to contamination on culture were present, guinea-pigs were also inoculated subcutaneously with the same sediment. The cultures were examined after twenty-four hours' incubation and in cases where only the cultural test had been made and where these now showed contamination a fresh sediment was prepared from the portion of tissue that had been frozen and was inoculated subcutaneously into guinea-pigs. Thus every precaution was taken to ensure the isolation of the infecting organism using as few guinea-pigs as possible on account of the limited space available for animal experiment.

The acid-concentration method, as recommended by Löwenstein (1924 (1)) and Sumiyoshi (1924 (1)), was also tried and found of use in case of autopsy material containing spore-bearing organisms, e.g., mesenteric glands; and while no extended series of comparisons was made, yet in 12 parallel experiments with the antiformin method already described the acid method appeared to be more lethal towards the tubercle bacilli. On the other hand, Corper and Uyei (1928), using the acid-concentration method and culturing the sediment so obtained on a specially prepared glycerin potato medium, found this method superior to all others for the isolation of tubercle bacilli/

bacilli by culture. Later Sweaney and Evanoff (1930) obtained even better results than Corper and Uyei by culturing these sediments on a special cream-egg medium.

In the case of 28 surgical specimens, cultures, and guinea-pigs where necessary, were inoculated directly with emulsions in distilled water of the tissues, this not being possible with the autopsy material on account of contaminating organisms.

(2) Isolation by culture.

Egg media, with and without the addition of glycerin (6 per cent.), were chosen as standard and were prepared as described by Wilson (1920) who used a tryptic digest of horse heart as the diluent instead of water as in the original Dorset (1902) formula, or in place of meat juice as in Petroff's medium (1915). Inspissation and the three subsequent sterilisations were carried out at 70°C. as recommended, higher temperatures being found to make the media less suitable for rapid growth of tubercle bacilli. The addition of 1 c.c. of a one per cent. watery solution of Grubler's crystal violet to every 100 c.c. of medium did not have any inhibitory effect on the growth of the tubercle virus while it helped in the detection of early growth, the pale colonies standing out against the greyish-blue background.

It/

It was very doubtful, however, if this 1 in 10,000 concentration of crystal violet prevented the growth of contaminating organisms which had not been destroyed by the antiformin treatment, and it was often omitted in the media used for sub-culture.

Both the ordinary egg medium (standard medium) and the glycerin egg were made at the same time from the same lot of switched eggs, tryptic broth being added in the one case and in the other the same broth with the addition of 6 per cent. pure glycerin. Both media were thereafter subjected to the same treatment during inspissation and subsequent sterilisations so that everything was kept as standard as possible in the preparation of the various batches of media, all new batches being tested with recently isolated strains which gave typical growths on the previous batch. Further, new-laid eggs from the same farm, with two exceptions, were always used; but even with these precautions the amount and rapidity of the growth varied much on the different batches of media and this appeared to be due to some factor beyond control, probably the eggs. The media were only used for cultural work up till six weeks after their preparation as after that time the growth produced was slower and not so abundant.

That results justified the choice of this modification of the original Dorset's media was demonstrated/

demonstrated in 28 parallel experiments using the standard medium made with tryptic digest and ordinary Dorset's egg medium, the tubes in each instance being inoculated from washed antiformin sediments. While little difference beyond slightly increased luxuriance of growth on the standard medium was noted with 15 eugonic strains in the primary cultures, yet a more rapid growth was obtained on the standard medium with 8 dysgonic strains. In 2 instances no growth resulted on the Dorset's media, while on the standard medium a slight growth of a dysgonic virus resulted in each of these. Inoculations with the remaining 3 sediments produced no growth on either medium after three months' incubation.

Four tubes of the above standard media and 2 of glycerin egg were each inoculated with three platinum loopfuls of the washed antiformin sediment or emulsions of fresh tissue prepared as described above, the inoculum being well rubbed into the surface of the media. In each of the culture tubes there was at least 0.5 c.c. of condensation water, and where this amount was not present sufficient sterile distilled water was added to make up the quantity so as to prevent drying of the media during the subsequent incubation. While more laborious, the inoculation of a number of tubes from each sediment is certainly an advantage as frequently growth occurred only/

only on a proportion of these and on rare occasions on only one. Further, often some of the tubes had a more characteristic growth than others, which helped greatly in typing the viruses according to their cultural characters and at the same time providing sufficient growth to allow of a rabbit being inoculated. The cultures were examined at the end of 24 hours' incubation to find if any contaminating growth had resulted and thereafter at weekly intervals when the rapidity, amount, and character of the growth were particularly noted in the case of primary cultures. For the first five to seven days' incubation the tubes were closed only with cotton wool plugs, but after this period a paraffin wax seal (a "hermedisc" as supplied by S. T. Gurr, London, was found most suitable) was placed on the top of the wool stopper which had previously been scorched in the flame before being pushed down below the level of the top of the tube. Whilst this procedure caused a certain degree of drying in the upper portion of the media, though not in the lower two-thirds on account of the condensation water, yet it helped growth considerably, probably on account of the fairly free access of air producing an early and abundant growth. Similar observations have been made by Novy and Soule (1925), who, working with cultures of one strain of human tubercle bacilli that had been in culture for/

for some time, noted that the growths obtained in cultures closed hermetically with sealing wax were less luxuriant than in those where a hole had been made in the wax with a hot platinum wire. They showed this to be due to an abundant supply of oxygen. In using this method, however, we found it caused much dehydration of the media after a period of 10 to 14 days. Later, however, Rockwell and Highberger (1926) maintained that carbon dioxide was a vital factor in the growth of the tubercle bacillus and not oxygen. Whatever gas is the essential one, the practical point remains that in tubes to which there is a free access of air in the first few days of culture a better growth is obtained than in those which have been sealed down immediately after inoculation.

Results.

Definite growth was generally visible in 5 to 14 days on the standard egg media, though with some strains, dysgonic types in particular, a longer period elapsed before colonies could be made out with the aid of a lens. Where no growth was visible at the end of 3 weeks the tubes were opened and a platinum loop was rubbed over the surface of the medium, this in some instances producing a fairly rapid growth, possibly by spreading/

spreading very fine colonies over the surface.

Isolation by direct culture with material derived from human sources was attempted with 263 antiformin sediments prepared from 226 post-mortem and 37 surgical specimens. In the case of other 28 surgical specimens the culture media were inoculated directly with emulsions in distilled water of fresh tissue. From the antiformin sediments one or more of the culture tubes showed growth in 170 (64.6 per cent.) instances, while from the fresh tissue a similar result was obtained in 12 (42.9 per cent.). From these results isolation after treatment with antiformin appeared to be more successful than by culture of emulsions of fresh tissue. This, however, was due to the large number of contaminations met with in the fresh specimens. Where tissues can be taken with absolute asepsis and where they are not the seat of a secondary acute inflammatory change, as is often the case with tuberculous cervical glands, emulsions of fresh tissue generally yield a very high percentage of positive results, particularly where the bacilli are numerous as, for example, in the tuberculous lesions of experimentally infected guinea-pigs. This method was adopted successfully in all instances in sowing cultures from lesions produced in 59 guinea-pigs inoculated from specimens in which direct culture from the/

TABLE I.

Methods used in the isolation of Human Strains.

A. Successful isolation by direct culture - 132 strains.

Source.	Number of bacilli in films.				Total.
	+++	++	+	-	
Antiformin sediment.	31	81	9	7	128
Fresh tissue emulsion.	1	1	2	-	4
Total	32	82	11	7	132

B. Successful isolation through guinea-pigs where direct culture had failed - 20 strains.

Result of culture.	Number of bacilli in films.				Total.
	+++	++	+	-	
Negative	-	-	3	6	9
Contaminated	-	2	7	2	11
Total	-	2	10	8	20

TABLE II.

Methods used in the isolation of Bovine Strains.

A. Successful isolation by direct culture - 50 strains.

Source.	Number of bacilli in films.				Total
	+++	++	+	-	
Antiformin sediment	7	22	10	3	42
Fresh tissue emulsion	-	5	3	-	8
Total	7	27	13	3	50

B. Successful isolation through guinea-pigs where direct culture had failed - 39 strains.

Results of Culture.	Number of bacilli in films.				Total.
	+++	++	+	-	
Negative	-	-	18	14	32
Contaminated	-	1	4	2	7
Total	-	1	22	16	39

the human tissue had failed. In the case of the experimental animal, however, the tissue for culture can be selected, whereas this is not often possible with human material obtained at operation.

In table I. the means by which the 152 human strains were isolated is shown. Of these 132 (86.8 per cent.) were obtained by direct culture; the remaining 20 in which the cultures failed were isolated through guinea-pigs. In table II the methods adopted in the isolation of the 89 bovine strains are summarised. Of the total bovine strains 50 (56.2 per cent.) were obtained directly by culture, while the remaining 39 were isolated from guinea-pigs as the cultural experiment in these cases had failed. It is evident from these results that, in general, human viruses were found to be more easily isolated by direct culture than bovine.

As will be seen from tables I and II the number of bacilli in the inoculated material was an important factor in the success or failure of isolation by direct culture. With 116 human strains where the bacilli were numerous or abundant in films of the inoculum direct cultures were positive in 114, while in 36 specimens where the bacilli were scanty or absent only 18 gave positive direct cultures. Similar findings were noted with 35 bovine strains where the bacilli were abundant or numerous in films of the inoculum, growth of tubercle bacilli/

TABLE III.

Isolation - Failure of direct culture from Human Material.

Source of Material.	Result of Culture.	No. of bacilli in films.				Result of culture.			Culture lost. Guinea-pig positive.
		+++	++	+	-	Lost.	Positive.	Per cent. lost.	
Thorax	Neg.	-	3	5	6	29	96	23.2	16
	Contam.	-	6	8	1				
Abdomen	Neg.	-	3	16	21	48	40	54.5	30
	Contam.	-	1	4	3				
Elsewhere	Neg.	-	-	2	4	8	4	66.7	3
	Contam.	-	1	1	-				
Surgical Antiformin	Neg.	-	1	6	-	8	30	21.1	5
	Contam.	-	-	-	1*				
Surgical Direct Culture	Neg.	-	-	-	5	16	12	57.1	5
	Contam.	-	3	7	1*				
						109	182	37.5	59

* Same specimen.

bacilli being obtained directly in 34 cases, though in 54 cases where the bacilli were scanty or absent only 16 (29.6 per cent.) yielded cultures. Accordingly, the difficulty of isolating bovine strains by direct culture was found to be much greater than with human strains when the bacilli were scanty or absent in films made from the material under investigation.

With 109 specimens the cultural experiment failed to isolate the infecting virus, no growth resulting in 72, while in the remaining 37 the cultures showed a heavy growth of contaminating organisms (table III). In 59 of these failures the infecting virus was isolated through the guinea-pig, leaving 50 specimens from which no strains were obtained. Failure to isolate by direct culture from tissues obtained at post-mortem and afterwards treated with antiformin was commonest in specimens derived from abdominal lesions (54.5 per cent.) and with those obtained from lesions in parts (mostly cervical glands) other than thorax or abdomen (66.7 per cent.). In the case of emulsions of fresh surgical specimens 57.1 per cent. were not successful on direct primary culture, due, as already noted, to the presence of contaminating bacteria in the tissue investigated.

Successful isolation by direct culture on the standard egg media used was thus found to depend largely on three factors:-

(1) the number of viable bacilli in the inoculum;

(2) the type of virus;

(3) the presence of other organisms; this factor depending largely on the site from which the material was taken, contaminations being found most frequently in cultures made from mesenteric and cervical glands.

(3) <u>Isolation through guinea-pigs</u>.

The guinea-pigs used in this investigation were always obtained from the same stock and were used for isolation of the infecting virus in selected cases as already noted. One guinea-pig was inoculated from each of these cases subcutaneously in the left groin with the same antiformin sediment, or in the case of surgical specimens with the same emulsion of tissue or pathological exudates as were used to inoculate the cultures. Both of these procedures were done on the same day except in 10 instances where on account of guinea-pigs not being available the washed sediments were frozen at -5°C. for periods ranging from 1 to 5 days in the case of 5 sediments which yielded human strains, and for 1 to 10 days in the case of the remaining 5 which contained bovine viruses. The freezing of the emulsions at this temperature and for these periods had little if any effect on the viability of the tubercle bacillus as in all 10 cases on inoculation into/

into animals a positive result was obtained. This agrees with the findings of Shope (1926) who noted that emulsions of both human and bovine tubercle bacilli lose little, if any, of their virulence when kept at refrigerator temperature for periods of 310 to 330 days. In other 5 cases where the cultures from the portion of tissue used for isolation had proved a failure or the inoculated guinea-pig had died prematurely, the halves of the specimens which had been put in the refrigerator at the commencement of the isolation experiments were antiformined and injected into guinea-pigs with positive results. In 2 of these cases which yielded human strains the tissue had been frozen for periods of 5 to 56 days, while in the other 3 which contained bovine viruses the period of freezing was from 5 to 17 days.

After inoculation the guinea-pigs were frequently examined for any palpable enlargement of the inguinal glands draining the site of inoculation, and as soon as this became marked they were killed. Animals in which this enlargement could not be distinctly felt were allowed to live at least 2 months, though some died before this date of intercurrent disease, and in some the antiformin treatment had not been sufficient to kill contaminating organisms and a diffuse fatal cellulitis resulted. This chiefly occurred in the case/

TABLE IV.

Isolation of strains through guinea-pigs.

A. Positive results in guinea-pigs - 93 strains.

Type.	Source.	Results of Direct Primary Culture.			Guinea-Pig Test Positive.	
		Positive.	Negative.	Contaminated.	Cult. Posve.	Cult. Lost.
Human	Antiformin sediment	14	9	11	14	20
	Fresh emulsion	-	-	-	-	-
Bovine	Antiformin sediment	19	27	7	19	34
	Fresh emulsion	1	5	-	1	5
Total		34	41	18	34	59

B. Negative results in guinea-pigs - 40 strains.

Time G.-Pig lived.	Source	Results of Direct Primary Culture				Total	
		Positive		Negative.	Contaminated.	G.-Pig Neg.	Cult. Posve.
		Hum.	Bov.				
A few days.	Antiformin sedt.	4	3	13	4	24	7
	Fresh emulsion	1	1	3	-	5	2
2 months or more.	Antiformin sedt.	3	-	6	-	9	3
	Fresh emulsion	-	-	2	-	2	-
Total		8	4	24	4	40	12

case of specimens taken from lesions in the abdomen where a tuberculous peritonitis with rupture of the bowel was present, or in the case of tracheo-bronchial glands related to cavities in the lung.

Results.

In table IV the results of isolation through guinea-pigs are summarised. Of the 133 guinea-pigs used for isolation 93 gave positive results, while of the remaining 40 some died shortly after inoculation, and others, though living two months or more, showed no lesion when killed. The same inoculum as had been used for the positive animals had given positive results on primary culture in 34 cases, so that in these much time was saved as it was not necessary to proceed with isolation from the lesions in the guinea-pig. In other 12 cases cultures were positive where the guinea-pigs had died prematurely or showed no lesion when killed. On the other hand, in the remaining 59 positive animals, the primary cultures made from the human tissues were negative in 41 and contaminated in 18 instances. Direct cultures were made in these cases from the guinea-pig lesions, the enlarged caseous inguinal lymph nodes being generally used, though in some cases where these had ulcerated and were likely to be contaminated the glands along the iliac vessels or/

or those in the lumbar region were taken. This resulted in the isolation of 20 human and 39 bovine strains. Of the 40 guinea-pigs which showed no lesion 29 of these had died shortly after inoculation before a lesion had had time to develop. In 9 of these the primary cultures had yielded a growth of 5 human strains and 4 bovine, while the corresponding primary cultures in the other 20 animals proved negative or contaminated. The remaining 11 negative animals had lived for about 2 months or more, 2 of these being inoculated with emulsions of fresh tissue which had also been used for direct culture with negative results, while 9 had been inoculated with washed antiformin sediments which yielded no growth in 6 and a very scanty growth in 3 cases. This observation, viz., a negative result in 3 guinea-pigs living 2 months or more and a positive result on culture, both being inoculated from the same material at the same time, seemed of importance and worthy of investigation. Park and Krumwiede (1910 (1)) have drawn attention to the fact that the presence of tuberculosis may be doubtful on macroscopic examination of inoculated guinea-pigs, and that cultural and further inoculation tests should be made, while Eastwood and Griffith (1916) remarked in a footnote:-

> "Tubercle bacilli of reduced virulence sometimes fail to produce macroscopic lesions in guinea-pigs though the bacilli inoculated survive and are recoverable from apparently normal tissues."

In the present 3 guinea-pigs, however, films and cultures made from the site of inoculation and from the various organs were negative for tubercle as well as other three guinea-pigs which were inoculated with suspensions of spleen and liver and tissue from site of inoculation from the first set of animals. On sub-culturing the scanty growths obtained in primary cultures from the original human material it was found that they were all of eugonic type. On intravenous inoculation of 0.01 mg. of culture into rabbits scanty small retrogressive lesions in the lungs and kidneys were the only lesions noted when the animals were killed over 2 months later. On subcutaneous inoculation of a similar dose into guinea-pigs these strains produced rather chronic tuberculous lesions. As such findings are unusual the protocols of these cases are given in full:-

Case 1772. Male, aged 2 years 5 months. No family history of tubercle. At post-mortem:- Pea-sized caseous focus in lower third anterior aspect right upper lobe and just under pleura; caseous tuberculosis of right superior tracheo-bronchial glands; numerous sub-acute miliary tubercles in lungs and scanty in spleen, none in liver or kidneys; tuberculous meningitis.

The right superior tracheo-bronchial gland mass was antiformined and no tubercle bacilli were seen in films/

films of sediment, 5 c.c. of which were inoculated subcutaneously into a guinea-pig which was killed 67 days later and showed no lesion. Films made from site of inoculation, liver, spleen and kidneys, showed no tubercle bacilli, and cultures made direct from liver, spleen, and the inguinal glands nearest the site of inoculation yielded no growth. An emulsion of liver, spleen and tissue from site of inoculation was made in distilled water and was inoculated subcutaneously into another guinea-pig which was killed after 62 days and showed no naked-eye evidence of tuberculosis. Films made from the various organs and site of inoculation were negative for tubercle bacilli.

Of the 6 cultures (4 on the standard egg media and 2 on glycerin egg) made from the original antiformin sediment one of the standard tubes yielded a few (4) colonies after 3 weeks' incubation, while the other 5 tubes showed no growth at the end of 2 months. The growth from the positive tube was sub-cultured on to 2 ordinary egg slants and produced a fairly heavy dry warty growth from which animals were inoculated. Further sub-cultures were made on glycerin potato, glycerin egg and glycerin serum, on which media a typical eugonic type of growth occurred, pigmentation being noted in the culture grown on glycerin serum.

On intravenous inoculation of a rabbit with 0.01 mg./

mg. of the first sub-culture and killing the animal at the end of 65 days, small (sago-grain sized) retrogressive lesions were found in the lungs and 2 small whitish foci were noted in one kidney, while the other organs showed nothing on naked-eye examination and films made from them were negative for tubercle bacilli. A guinea-pig was also inoculated subcutaneously in the left groin with 0.01 mg. of culture and killed after an interval of 60 days, and showed a small local lesion at the site of inoculation, while the left inguinal and iliac glands were enlarged and caseous and a few caseous points were present in the right inguinal lymph nodes and in the spleen and liver.

Case 1820. Female, aged 17 months. No family history of tubercle. At post-mortem:- caseous focus in right upper lobe, right broncho-pulmonary and superior tracheo-bronchial glands markedly caseous and to a less extent the glands along the right and left side of trachea. Generalised sub-acute miliary tuberculosis with tuberculous meningitis.

The right superior tracheo-bronchial gland mass was antiformined, but no tubercle bacilli were found in films of sediment. A guinea-pig was inoculated subcutaneously with 5 c.c. of the washed sediment and killed 91 days later, but no lesion was noted anywhere and films made from the various organs and site of inoculation showed no/

no tubercle bacilli. Cultures on egg media made from splenic and liver pulp yielded no growth at the end of 9 weeks. Portions of the liver, spleen and tissue from the site of inoculation were introduced under the skin of the abdomen of another guinea-pig which was killed after a period of 59 days, but no evidence of tubercle was found either on naked-eye examination, or in stained films made from the organs.

Only one of the six cultures inoculated from the antiformin sediment yielded a growth consisting of 2 colonies which at the end of 4 weeks were sub-cultured on to two tubes of egg media. From these tubes after growth had taken place sub-cultures were made on glycerin potato, glycerin egg, and glycerin serum, on all of which a typical, eugonic, slightly pigmented growth occurred.

From the second sub-culture a rabbit was inoculated intravenously with 0.01 mg. and killed 64 days later when very small scanty caseous lesions were noted in lungs and a few in the kidneys. At the same time as the rabbit was inoculated a guinea-pig received a similar dose subcutaneously and was found dead 49 days later, and on examination a small caseous local lesion was present at the site of inoculation, while the related inguinal glands showed moderate caseous foci. In addition there was a localised caseous peritonitis under the lesion at site of inoculation and a few caseous foci in spleen.

Case 1989. Female, aged 7 months; history of tuberculosis (type not specified) in two maternal uncles.
At post-mortem:- small caseous focus in lower third of right upper lobe a short distance under anterior aspect; right superior tracheo-bronchial glands caseous as well as the glands on the right side and to a less degree those on the left side of trachea. Numerous miliary tubercles in the lungs, scanty in liver and spleen, none in the kidneys, and no meningitis.

The right superior tracheo-bronchial gland mass was antiformined, but no tubercle bacilli were seen in films of the sediment. A guinea-pig inoculated with 4 c.c. of the washed antiformin sediment subcutaneously in the left groin died 58 days later and no lesion was noted at post-mortem at the site of inoculation or in any of the organs, while films made from the left inguinal glands, spleen and liver were negative for tubercle bacilli as also were cultures made from emulsions of liver and spleen. A guinea-pig inoculated with an emulsion of spleen and tissue from site of inoculation was killed 62 days later and no lesion was noted in the region of the inoculation or in the neighbouring glands, while films from these sites as well as from liver and spleen showed no tubercle bacilli.

One of the six tubes inoculated from the original antiformin sediment yielded a growth of 3 colonies which on sub-culture on 2 ordinary egg tubes grew well and on further/

further sub-culture on egg, glycerin potato and glycerin egg, produced a typical warty dry eugonic type of culture.

A rabbit was inoculated intravenously with 0.01 mg. of the first sub-culture and killed 66 days later, when scanty small caseous lesions were noted only in the lungs, while a guinea-pig inoculated subcutaneously at the same time with a similar dose and killed 79 days later showed a small area of caseation at the site of inoculation, while the inguinal and iliac glands in relation were almost entirely caseous, and some caseous foci were noted in the spleen and liver.

From the results of these experiments the following conclusions can be drawn:-

(1) That either there were no tubercle bacilli, or that these organisms were dead in the inoculum used for the guinea-pig. This was unlikely as the cultures which gave positive results were inoculated at the same time from the same twice-washed antiformin sediment as was used for injection into the guinea-pig, and further the amount of the inoculum for the animal was many times larger than that used for culture.

(2) That the animals had not been allowed to live long enough after inoculation for a lesion to be produced. This in some respects would have been a just criticism, but/

but in addition to no lesion being produced the organisms inoculated had not multiplied or had been killed off, as films made from the tissues of the guinea-pig showed no tubercle bacilli, and negative results were also obtained in cultures and on further inoculation of guinea-pigs.

(3) That the bacilli isolated were of avian type. The nature of the lesions in the three children was very unlike that produced by the avian type of bacillus, and further all three children gave positive von Pirquet reactions to human tuberculin and in two of them to bovine tuberculin also. Further, the cultures isolated were typical of the human types of virus though admittedly the distinction between some human and avian types of culture may be difficult. The fact that guinea-pigs were able to be infected with as small a dose as 0.01 mg. of culture inoculated subcutaneously was also against the avian hypothesis as well as the results in the rabbits which were typical of the reaction following the inoculation of the human type of virus.

(4) That these 3 strains of the tubercle virus were of low virulence and that the guinea-pigs used were more resistant to infection than usual so that after inoculation the scanty organisms were killed off before they had time to adjust themselves to their new surroundings and multiply. This/

This would appear to be a likely explanation in view of the fact that on inoculation of other animals (rabbits and guinea-pigs) with 0.01 mg. of these cultures only minimal lesions were produced. This is in accord with the work of Sumiyoshi (1924 (2)) who, while studying the virulence of 30 strains isolated from sputum, found that 2 of them produced no lesion in guinea-pigs which were inoculated with emulsions of culture, while the other strains varied much in their virulence for the guinea-pig. Löwenstein (1924 (1 & 2)) made similar observations in the case of 2 out of 30 strains isolated from sputum as well as in 3 out of 14 obtained from urine, and both these authors suggested the possibility that these strains might have been of avian type. Schmidt (1927), working with tuberculous sputa, found that on one occasion out of 40 the guinea-pig test was negative, while cultures on Lubenau's egg medium and glycerin potato were positive in the case of a specimen which showed no tubercle bacilli in films. His experiments, however, are not quite comparable to our own or Sumiyoshi's and Löwenstein's, as, although animal and culture were inoculated from the same sample of sputum, yet the portion used for culture was subjected to a different treatment to that used for guinea-pig inoculation, the inoculum for the guinea-pig being washed four times. In our experience, unless the washing water is pipetted carefully off without disturbing the/

the top layers of the sediment in which the tubercle bacilli are often numerous, there is a likelihood of the organisms being carried off in the water, and this is a possible explanation of this worker's result. Morse and Braasch (1927) had a similar experience to ours with urinary sediments, obtaining negative results in 2 out of 45 with the animal test, 2 guinea-pigs being inoculated from each specimen, one intraperitoneally and the other subcutaneously. On the other hand, Levinthal (1927) produced tuberculosis in guinea-pigs (living for 31 to 46 days after inoculation) with a human strain, isolated from sputum, with from 1 to 3 bacilli, only one animal which lived for 21 days giving a negative result. The Royal Commission (1911 (3)) caused fatal tuberculosis in two guinea-pigs which died 184 and 188 days after having been inoculated subcutaneously with 0.00001 mg. of human cultures, i.e., about 45,000 bacilli according to the methods adopted by the Commission for estimating the dose of the inoculum (1911 (4) and 1907 (2)). Fraenkel and Baumann (1906) in an extensive investigation into the virulence of various strains of tubercle bacilli obtained from human sources (chiefly sputa) found very little variation in the sensitiveness to infection on the part of guinea-pigs even with doses as low as a hundred millionth part of a milligram (approximately one bacillus according to their estimation), and during the/

the 6 years their experiments were in progress there was no appreciable loss in virulence for the guinea-pig of the strains with which they had been working. Some of these results are not in agreement with the experiments of Thöni and Thaysen (1916), who, on inoculation of a series of guinea-pigs with from 10 to 76 highly virulent tubercle bacilli, obtained from a human source, found that only one which had received 71 organisms developed tuberculosis. In a second series of experiments with cultures of 3 strains of bacilli, all of which were obtained from human lesions and 2 of which were of high virulence, they obtained negative results in 22 guinea-pigs, some of which were inoculated intraperitoneally and others subcutaneously with from 99 to 343 bacilli.

There is no record, however, in any of these worker's papers, apart from those of Sumiyoshi and Löwenstein, of cultures being made at the same time and from the same material as was used to inoculate the guinea-pigs, and while tubercle bacilli were seen in films, yet such is no proof of their viability. The literature contains many examples of negative results of culture and animal experiment, both being inoculated from the same material, chiefly emulsions of caseous glands, in films of which tubercle bacilli were demonstrated, and the conclusion/

conclusion justly drawn in these cases was that the tubercle bacilli were dead (Royal Commission, (1911 (2)); Weber (1906); Griffith, A. S. (1914 (3) and 1929); Eastwood and F. Griffith (1924 (2)).

Thus the important question arises as to whether the guinea-pig or culture is the more reliable test for the presence of tubercle bacilli in a specimen. In this connection the observations of Corper and Uyei (1929) are of much practical importance. These workers found the cultural test as sensitive as the guinea-pig when they used a special potato medium on which was sown the sediment obtained by the acid-concentration method already mentioned. Sweaney and Evanoff (1930), also using an acid-concentration method and a cream-egg medium, found that the culture gave slightly better results than animal inoculation, as in 8 of their specimens the guinea-pig test was negative while cultures were positive, and in only 4 cases was the reverse found, viz., the animal test positive and cultures negative. On the other hand, Lutz (1929) found that the animal test was more reliable than culture when he made the inoculation into the lymph nodes behind the guinea-pig's knee, though he admitted that when using the subcutaneous and intraperitoneal methods he sometimes obtained a positive result on culture and a negative one in the animal. In the present investigation culture/

culture has been found a useful addition to the guinea-pig for isolation, the animal experiments being regarded as more secure, particularly in the case of bovine strains. The more practical procedure in the case of specimens for diagnosis would appear to be to use culture as well as guinea-pig, as by adopting both methods there is a greater security with the possibility, if the culture is positive, of an earlier diagnosis.

SECTION II.

Determination of type of virus.

SECTION II.

Determination of type of virus.

(1) By morphology.

The morphological appearances of the infecting bacilli were always noted either in the films made directly from the tissue or from the antiformin sediment. Of the 152 viruses which were ultimately typed as human 119 (78.3 per cent.) were long and beaded. With the bovine viruses more variation was noted; only 52 (58.4 per cent.) of the 89 isolated were short and showed no suggestion of beading. Accordingly, little importance was attached to the morphological appearances of the viruses in the films made as described above. In the case of films made from primary cultures the variations in size and form were even greater. Similar observations to these have been made by the Royal Commission (1907 (1)), by Park and his co-workers (1910 (2)), and by Wolbach and Ernst (1903). True branching, however, was never seen in any of the films made directly from the infected tissues.

(2) By culture.

The character and, in particular, the amount and rapidity of growth, especially on media containing glycerin, were used as criteria to distinguish between eugonic and dysgonic/

dysgonic types, and as primary cultures from antiformin material often yielded only scanty growth, early sub-cultures were always made, at least two tubes of each type of media used in the particular experiment being inoculated. For purposes of classification the cultural appearances of the primary culture and first four sub-cultures were used, the best growth in any tube of this series being taken as standard. The period of incubation was 4 to 6 weeks, when transfers were made from one standard egg medium to another or to media containing glycerin. With some of the more slowly growing cultures in which only a few colonies were present transfers were made at the end of 2 to 3 weeks, as this more rapid sub-culturing often produced, in a shorter period of time, the maximum growth a particular strain was capable of. In cultures put up for the purpose of showing pigmentation the incubation period was generally longer. With some human strains typical growth characteristics sufficient for a diagnosis as to type were produced early, and it was not considered necessary to proceed with all the 4 sub-cultures. In a few cases more than 4 transfers were made in an attempt to elicit more definite cultural characters. It was noted, however, that with repeated transfers the distinction in many cases between the two types became less marked, and while in sub-cultures made from ordinary egg/

egg media to these containing glycerin differences could still be made out, yet these were not as evident as in the earlier sub-cultures. At the beginning of this investigation, before much experience had been gained, this offered some difficulty, so it was decided, for purposes of classification and uniformity, to adhere to the cultural characters, etc., of the primary growth and first 4 sub-cultures of each strain. Park and his co-workers (1910 (3)) adopted a similar plan successfully in their extensive investigation. There is little doubt, however, that this difficulty in the early stages of the research was enhanced by slight variations in each batch of culture media although all precautions were taken to ensure uniformity. As experience was gained these variations as judged by the results of culture became less, except in the case of serum media and to a less extent ordinary egg media. With these the variable factor appeared to be beyond control. As already noted, glycerin egg media as well as tubes of standard egg media were always inoculated with the material to be cultured. When the results were not sufficiently diagnostic with these two media in the primary cultures and first two sub-cultures, glycerin potato and sometimes glycerin serum were also inoculated in addition to the occasional use of the wax experiment described later. The precaution was/

was always taken to make the transfers to the glycerin media from cultures on ordinary egg or serum media so as not to allow any adaptation on the part of the organism to this substance. For purposes of classification a strain was considered eugonic when the growth on media containing glycerin was better or at least equal to that on ordinary media. In this respect glycerin potato and glycerin serum were found to give sharper results than the glycerin egg medium. Unfortunately, it was not possible to culture all the strains isolated on all the media available. More marked cultural differences in the case of typical strains might have been brought out if this had been done, for with definite cultural variants several media helped greatly to define more clearly growths showing intermediate characters on the standard egg and glycerin egg. The cultures of human strains on glycerin media were largely used for inoculation as the growth was found to be more easily emulsified than that on media without glycerin.

The presence of pigmentation has been regarded as very characteristic of human strains by Griffith (1916 (1)) who used inspissated bovine serum of a golden yellow colour, the serum being always drawn off aseptically from the same cow. In the present investigation inspissated bovine serum was prepared on twelve occasions, but only 3 batches gave the results described by that author, and these/

these were conserved as long as possible to test doubtful cultures; the remainder with known human strains produced greyish or pale cream non-pigmented colonies. There is little doubt, however, that the failure experienced with this medium was due largely to the fact that the serum could only be obtained from the slaughter-house and different batches varied much in colour. Accordingly, glycerin potato medium was largely used for pigment production and was found useful in this connection, at the same time giving fairly constant results, though Park and his co-workers (1923 (4)), using a similar medium, found that pigmentation was not solely confined to human strains. In our experience some of the more rapidly growing bovine strains produced pinkish colonies on glycerin egg media but never on glycerin potato.

By accident it was noted that when the paraffin wax which was used to seal the tubes ran down over the surface of the media where early growth was visible a more rapid growth occurred along the edge of the wax, generally with marked pigmentation in the case of the human strains. On repeating this observation by running from a pipette the ordinary melted (52°C.) embedding paraffin wax down the centre of an egg slant that had been inoculated 7 to 10 days previously with a eugonic strain and re-incubating, there occurred along the edge of/

of the wax a general heaping-up of growth, so that after about 2 months the wax streak formed the base of a trough, the walls on either side of which were composed of a fairly continuous line of irregular heaped-up warty yellowish-red growth. With bovine strains there was also an increase in growth along the edge of the wax, though it was not so marked as in the case of human strains. Beeswax, spermaceti, stearin, palmitin, and agar were also tried, but the best results were obtained with the ordinary paraffin wax, little change in the character of the growth being produced with the agar. This method, in addition to being useful as a stimulus to some of the more slowly growing human viruses, also helped with observations on pigmentation as well as providing a ready and simple means of obtaining abundant growth for experiments with mice where large doses (1 mg.) of tubercle bacilli were required to produce lesions. The effect of the waxes would appear to be due to their providing an area where the water content is low, substances such as paraffin wax and beeswax being more efficient than palm oil, palmitin, or agar-agar, which are more miscible with water. When these various waxes were incorporated in the medium by making an emulsion with the warm egg-broth mixture and then rapidly inspissating, little difference was observed in the amount of growth as compared with that on ordinary egg media.

As/

As early as possible a preliminary division into eugonic and dysgonic types was made so that rabbits could be inoculated from early cultures to avoid any possible attenuation of the virus. Although Cobbett (1917), who has collected together the various experiments of the Royal Commission on this subject, noted that for rabbits there was only a negligible degree of attenuation of the bacilli after a period of 2 years in culture, yet Park and his co-workers (1923 (5)) found that two of their bovine strains showed early decrease in virulence when kept on culture media. We have also noted that in the case of the mouse some human and bovine strains rapidly lose virulence. The inoculation of animals at this stage also allowed an early correlation to be made of the results of the animal and cultural experiments, and where these did not correspond, further sub-cultures could be made, or the virus re-isolated from the rabbit tissues, or other rabbits inoculated from sub-cultures.

Results of culture.

Human strains.

To consider first the 152 strains classified as human on account of the results of cultural and animal experiments. Of these,134 showed typical eugonic characters in transfers made from the primary cultures or one of/

of the first 4 sub-cultures on the standard egg medium to media containing glycerin as follows:-

134	65	in	transfers	from	primary cultures and 1st sub-cultures		to glycerin egg	
	32	"	"	"	2nd	"	"	" "
	24	"	"	"	3rd	"	"	" " " potato
	13	"	"	"	4th	"	"	" egg " potato " serum

In transfers made from the third and fourth sub-cultures other glycerin media were used in addition to glycerin egg. Once typical eugonic characters were noted no further cultural observations were made.

With the remaining 18 strains even after four sub-cultures the eugonic characters were less prominent. The growth of these on glycerin egg and glycerin serum was only slightly better than that on ordinary media, while that on glycerin potato was equal or slightly less. In this respect the cultural characters of these strains corresponded with the growths given by the more luxuriantly growing bovine strains. In 12 of these, however, definite yellow or reddish pigment was produced on inspissated ox serum or on glycerin potato. In other 4 on re-isolation from the rabbit the strains grew more luxuriantly, while with/

with the remaining 2 strains the most noteworthy feature was that no pigmentation was obtained on glycerin potato on which the growths consisted of a thin greyish film. On re-isolation of these 2 strains from rabbits which showed very typical human reactions no great difference in the character of the growths was noted, even though repeated sub-cultures on egg media at intervals of four weeks were made in the one case to the fourteenth generation and in the other to the twelfth. On transfers then being made to egg, serum, potato, and agar, all of which contained glycerin, the growths on the first two media were better than that on ordinary egg, while on the serum medium yellowish pigment was produced. On the other two media the growth produced still consisted of a very slightly raised dull whitish layer of scanty growth. This character, viz., minimal growth on glycerin potato, had remained even after animal passage and prolonged sub-cultivation. These two strains, both of which were isolated from thoracic lesions, conform to the description of the dysgonic variety of the human virus given originally by A. S. Griffith (1916 (2)) and by Eastwood and F. Griffith (1916).

Of the 152 human strains isolated 94 were also tested for type by inoculation of rabbits. These included all the strains which only showed typical eugonic characters/

characters in transfers from the fourth sub-culture as well as the 18 strains whose eugonic characters were ill-defined. Of the 58 strains not tested for virulence by animal experiment 4 had been isolated from secondary lesions and as their cultural characters were similar to these obtained from the primary site of the disease and which were tested for virulence no animal test was considered necessary. The remaining 54 strains produced typically eugonic growths in the transfers made from the primary, first, second or third sub-cultures and all had produced definite pigment. On account of these very distinctive cultural characteristics we did not proceed in the later stages of the investigation to type such strains by animal experiment. All these 54 human strains were obtained from thoracic lesions which had caused death.

Bovine strains.

All of the 89 bovine strains isolated were carried through all four sub-cultures, and of these 77 remained typically dysgonic throughout, giving either scanty or no growth on sub-cultures made on glycerin egg media. With other 11 strains the growth on glycerin egg media in the later transfers was equal to that on the standard egg, though in all of them the growth was either sparse or failed in the sub-cultures made from the primary cultures. In transfers made to glycerin egg from the first and second/

second sub-cultures the amount of growth closely approached that on the standard egg medium though the rate was slower. This was more noticeable in cultures made from the second sub-cultures than from the first. With transfers made from the third and later sub-cultures on egg medium, glycerin potato was used in addition to glycerin egg, and with these 11 strains the growth on the latter equalled that on the ordinary medium, while that on glycerin potato was not as luxuriant as that on the ordinary egg. Thus it is evident that the growth characteristics of these 11 bovine strains did not differ much from that given by the more slowly growing human strains. On more critical examination, however, the colonies on the glycerin media were found to be smaller than those on ordinary media, and as compared with human types the growths were more moist and not so adherent, while in the first two weeks of culture the growths on the glycerin media showed a distinct lag behind that on the ordinary media, whereas in the less luxuriant human strains this was not a marked feature. In all of them, however, no definite yellowish pigment was produced on glycerin potato or inspissated serum though cultures were kept in the incubator for some months to allow time for this to occur. With the remaining bovine strain it was difficult in the light of the results of animal experiments to decide into what category the growth should/

should be placed. This strain had been isolated through antiformin from a tuberculous hip-joint. In primary culture a moderate growth was obtained on the standard egg media while on glycerin egg inoculated at the same time a few colonies developed. In the first sub-cultures from the standard medium the growth became more abundant both on ordinary and glycerin egg, the growth on the latter being almost equal to that on the ordinary medium. From the first sub-culture transfers were made to egg and potato, both of which contained glycerin, and on the first of these media an abundant creamy growth resulted which was as luxuriant as that on ordinary egg, while in the case of the glycerin potato the growth was not so profuse. No definite yellowish pigment was produced on any of the media. This strain was accordingly classified as human, but when the result of animal experiment became available it was found that 0.01 mg. of culture intravenously had produced an extensive generalised miliary tuberculosis, the animal dying 48 days after inoculation. The possibility then suggested itself that the culture used for inoculation consisted of a mixture of human and bovine strains and the strain was re-isolated from the kidney of the rabbit, while a sub-culture of the original culture was plated out on glycerin egg. The re-isolated strain corresponded in all its cultural reactions to that given by the original, while in/

in the plates all the colonies appeared identical. There still remained, however, the possibility that this culture was particularly virulent for the rabbit when inoculated intravenously and accordingly 5 mg. of a sub-culture of the original growth used for inoculation was injected subcutaneously into a rabbit which died in 6 weeks of a generalised miliary tuberculosis. Thus this strain gave a bovine reaction in the animal and was more eugonic in culture than the other bovine strains investigated. Park and Krumwiede (1923 (6)) and Griffith (1919) have reported similar luxuriantly growing bovine strains, though in the case of the former workers the cultures had been transferred from one glycerin egg culture to another and hence the strains had been given an opportunity to adapt themselves to glycerin, and this probably accounts for the increase in the amount of growth.

All of these bovine strains with 2 exceptions were also typed by the results obtained from the inoculation of rabbits. In the 2 exceptional cases the strains were obtained from secondary lesions, and as their cultural characters corresponded with those isolated from the primary site of the disease and which were tested for virulence no animal tests were made with them.

(3) <u>By results of inoculation of rabbits</u>.

Of the 241 strains isolated, 54 human strains, as already/

already noted, were typed by culture alone, while in 6 cases (4 human and 2 bovine), in each of which 2 culturally similar strains were isolated from the primary and secondary lesions, only those obtained from the former were tested by inoculation of rabbits; the remaining 181 strains (94 human and 87 bovine) were typed both by animal and cultural experiments.

Animals of not less than 1,200 grams were used, and while variation in size made little difference to the results, yet as far as possible the smaller animals were used for testing the eugonic strains. The ordinary English and Dutch types of rabbit were found most suitable for the work as more highly-bred species, e.g., the blue beveran, proved less resistant to infections with human strains. The intravenous method of inoculation was chosen chiefly in view of the fact that quicker results were expected, and also, as the space in which the animals were kept was small, ulceration of the local lesion produced by subcutaneous inoculation would have been likely to contaminate the surroundings and so spread the infection to other animals. As is described later, even with the extensive, though not exclusive, use of the intravenous method, probably one case of crossed infection occurred. The dose chosen was 0.01 mg. and this was adhered to throughout with all dysgonic cultures, while/

while in the case of 40 eugonic cultures the animals received doses of 0.1 mg. Only one animal was inoculated from each strain, and where the results did not prove satisfactory, e.g., the reaction in the rabbit not corresponding to the type of culture, the animal experiment was repeated using either the intravenous route and same dose, or more often the subcutaneous with a dose of 5 or 10 mg. By this means any increased susceptibility on the part of individual rabbits was checked, and also the number of animal experiments was reduced to a minimum to suit the space at our disposal, while the use of the subcutaneous method of inoculation with its attendant risk of dissemination was avoided as far as possible.

The material for inoculation was taken, in the majority of cases, from the primary culture or from the first, second, or third sub-cultures on the standard egg media, though as already observed in the case of most of the eugonic strains the glycerin egg cultures were used as these were more easily emulsified. Cultures of 3 but not more than 4 weeks' duration were used so as to have the number of viable bacilli as nearly equal as possible in each inoculation experiment. The dose was estimated by weighing on a chemical balance a small portion of culture which was picked off with a platinum spatula and placed at the bottom of a weighed sterile tube, and with some experience small amounts weighing between 0.5 and 2 mg./

mg. could be picked off. The weighed amount was carefully triturated for 5 minutes with a glass rod rounded at the end when a few drops of water were added from a burette and the mixture thoroughly stirred so as to form as homogeneous an emulsion as possible and the whole was then diluted with water so that 1 c.c. would contain 0.1 or 0.01 mg. of culture, which was used for inoculation. As the coarser particles were allowed to settle before inoculation, the 1 c.c. used contained rather less than 0.1 or 0.01 mg. and in three estimations on different emulsions of bovine bacilli an average bacterial content of 21 million was found in doses representing 0.01 mg., so that in respect of the number of bacilli the doses employed were smaller than those used by the workers of the Royal Commission (1907 (2)). The doses were injected slowly into the ear vein of the rabbits so as to ensure an even distribution of bacilli.

Any animals surviving at the end of at least two months were killed. While, if space had allowed, it might have been more convincing to have let the surviving animals live for a longer period, yet little difficulty was encountered by adopting this time limit, as most of the animals inoculated with bovine strains were already dead of a generalised tuberculosis. In the few cases where these bovine infected animals survived for two months/

months, the lesions were generally easily distinguishable from those in rabbits inoculated with a human strain and killed after the same length of time. Where animals died six weeks or less after inoculation with a eugonic strain, either from the tuberculous lesions or intercurrent disease, the experiment was repeated. From all the inoculated animals smears were made at post-mortem from the organs and examined for tubercle bacilli. This was of much help in cases where there was difficulty in distinguishing between pseudo-tuberculosis or coccidiosis and lesions due to the inoculation of the tubercle bacillus, though the former lesions for the most part were confined to the liver and spleen and were whiter and rounder than foci of real tuberculosis.

The standards adopted for classifying the reactions in the rabbit as characteristic of a human or bovine virus were those described by the Royal Commission (1911 (5)) and need not be repeated in detail here. Briefly, these workers found that nearly all bovine strains produced a rapidly generalised acute miliary tuberculosis with marked pulmonary lesions, while in the case of most human strains the lesions were retrogressive, being confined to lungs and kidneys, and did not appreciably affect the health of the animal.

Results of inoculation of rabbits.

Human strains.

Dose = 0.1 mg. intravenously.

In the case of 40 eugonic cultures isolated in the earlier part of this work, 0.1 mg. was inoculated intravenously, one rabbit being used for each culture. Of these 4 commenced to emaciate soon after the inoculation, dying at periods of 20 to 32 days, when it was found that the lungs showed extensive consolidation due to numerous large areas of caseation, while the kidneys showed fairly numerous small caseous nodules chiefly in their cortical regions, and in 2 of the cases a few small caseous foci were found in the spleen (table V, first part). On repeating the intravenous inoculation in other rabbits from sub-cultures of those employed for the first series of inoculations, but using 0.01 mg. as the dose, 3 of the animals were killed after 65 days or more and showed minimal pulmonary and renal lesions. The other one died at 54 days with scanty pea-sized caseous lesions in the lungs, a few caseous points being noted in the tracheal glands and in the cortical areas of the kidneys, but no evidence of tuberculosis elsewhere, though in addition there was extensive pseudo-tuberculosis of bowel, spleen, and liver which was verified histologically. Of the remaining 36 animals/

TABLE V.

Rabbits showing unusual reactions when inoculated intravenously with 0.1 mg. of human viruses.

TABLE V. Rabbits showing unusual reactions when inoculated intravenously with 0.1 mg. of human viruses.

i.v. = intravenous inoculation; s.c. = subcutaneous.

No. of Case.	Type of Case.	Culture used.	Dose mg.	Route	Post-mortem findings.
1498	Male, 5 months. Caseous-walled cavity in right middle lobe. Tuberculous broncho-pneumonia - caseous thoracic glands.	2nd Sub.	0.1	i.v.	Dead 20 days. Emaciation marked. Lungs voluminous; large confluent caseous areas. Caseous foci in tracheal glands. Caseous points in kidneys.
		3rd Sub.	0.01	i.v.	Killed 68 days. A few small caseous foci (pin-head size) in lungs, a few miliary tubercles in kidneys.
1702	Male, 13 wks. Tuberculous broncho-pneumonia and thoracic glands; sub-acute generalised miliary tuberculosis.	3rd Sub.	0.1	i.v.	Dead 28 days. Blue beveran rabbit. Marked emaciation. Large confluent caseous areas in lungs. Small caseous foci in tracheal glands Caseous points in kidneys and spleen.
		4th Sub.	0.01	i.v.	Dead 54 days. Very scanty pea-sized caseous lesions in lungs; a few small caseous foci in tracheal glands and kidneys. Extensive pseudo-tuberculosis of liver, spleen and caecal region (microscopic exam.)
1624	Male, 21 mths. Tuberculous meningitis & masses in cerebellum. No other lesion found.	1st Sub.	0.1	i.v.	Dead 32 days. Moderate emaciation. Lungs voluminous & many large confluent caseous areas; small caseous foci in tracheal glands & kidneys. 2 minute caseous foci in spleen
		2nd Sub.	0.01	i.v.	Killed 65 days. A few small pin-head size lesions in lungs and very scanty similar lesions in kidneys.
S4	Male, aged 8 yrs. Operation for chronic appendicitis - caseous mesenteric glands.	3rd Sub.	0.1	i.v.	Dead 32 days. Blue beveran rabbit. Several large caseous areas in all lobes of lungs, a few caseous points in kidneys.
		4th Sub.	0.01	i.v.	Killed 70 days. Numerous chronic fibrotic lesions in lungs. Nil in kidneys.
1547	Male, aged 11½ yrs. Chronic caseous foci in lungs, chronic pleurisy, tuberculous thoracic glands, tuberculous meningitis.	1st Sub.	0.1	i.v.	Dead 60 days. Lungs voluminous - several confluent caseous areas; caseous points in tracheal glands, scanty miliary foci in spleen and kidneys.
		3rd Sub.	0.01	i.v.	Killed 68 days. Moderate number of small caseo-fibrotic lesions in the lungs only.
1634	Male, aged 2 yrs. 1 mth. Limited p.m. Tuberculous meningitis.	2nd Sub.	0.1	i.v.	Killed 65 days. Lungs, Moderate number of small discrete caseous foci, tracheal glands enlarged & ? caseous points. Few miliary lesions in kidneys.
1509	Female, aged 14 mths. Tuberculous broncho-pneumonia, cavities, tuberculous thoracic glands.	1st Sub.	0.1	i.v.	Killed 67 days. Lungs, scanty small (lentil size) caseous lesions and some chronic fibrotic lesions. Tracheal glands enlarged. Few miliary foci in kidneys.
1692	Male, aged 12 mths. Tuberculous broncho-pneumonia, cavities; generalised sub-acute miliary tuberculosis; tuberculous meningitis.	2nd Sub.	0.1	i.v.	Killed 67 days. Lungs, two large pea-sized caseous areas in left caudal lobe, smaller discrete caseous foci elsewhere; caseous points in tracheal glands. Few miliary points in kidneys
S.9	Male, aged 9 mths. Tuberculous osteitis of femur.	3rd Sub.	0.1	i.v.	Killed 68 days. Three large foci of caseation in lungs; tracheal glands enlarged & slightly caseous. A few miliary lesions in kidneys.
1569	Female, aged 9 months. Caseous lung focus. Tuberculous thoracic glands - generalised miliary tuberculosis, tuberculous meningitis.	2nd Sub.	0.1	i.v.	Killed 71 days. One pea-sized caseous lesion in right caudal lobe, numerous chronic fibrotic lesions. Nil in kidneys or tracheal glands.

animals, 8 died from 49 to 60 days after inoculation, while the remainder were killed after 65 days or more. All of these 36 animals had remained in good health. At autopsy the lesions were minimal in the lungs and kidneys with 6 exceptions, in 5 of which, killed between the 65th and 71st days after inoculation, the pulmonary lesion consisted of several confluent areas of caseation or a few large caseous foci with in some animals caseous foci in the tracheal glands and scanty miliary lesions in the kidneys (table V, second part). Although the lesions in the lungs in these cases were more severe than usual for human strains and were not unlike those produced by some of the bovine viruses, yet there was no generalisation, though ample time had been allowed for this to occur. Further animal tests in these 5 cases were not considered necessary as the cultures with which these animals were inoculated were all typically eugonic. In the remaining case (1547), which died on the 60th day, there was a fairly extensive confluent caseous lesion in the lungs with caseation in the tracheal glands and a few miliary foci in the kidneys and spleen. The inoculation test was repeated in this case using 0.01 mg. of a sub-culture from the original tube used for inoculation, and when the animal was killed 68 days later minimal lesions were found only in the lungs. Thus in 5 rabbits out/

out of the 40 inoculated with 0.1 mg. the lesions produced were severe and approached those given by the bovine type, so that for a definite diagnosis another inoculation test had to be made, and it is to be noted that 2 of the animals were of the blue beveran variety.

While we had hoped to bring out more sharply the difference in the lesions produced in the rabbit by the two types of bacilli, using, in the case of human strains, a dose (0.1 mg.) 10 times greater than with bovine (0.01 mg.) yet the above results made it clear that the larger dose in the case of the former strains gave rise to a considerable number of lesions which were severe and in some cases equal to that given by the bovine type of bacillus and not in accordance with the cultural findings. This difficulty was not experienced to the same extent on repeating the experiments using 0.01 mg., and so for the remainder of the investigation this dose was used for both strains.

Dose = 0.01 mg. intravenously.

Of 54 animals receiving this dose one (1796) died in 30 and another (S.20) in 35 days. Both of these which were of the blue beveran type were inoculated with typical eugonic cultures and showed extensive caseous pulmonary lesions, with involvement of the tracheal glands in one case, while in both the only other lesion was a few pin-head size whitish foci in the kidneys/

TABLE VI.

Rabbits showing unusual reactions when inoculated intravenously with 0.01 mg. of human viruses.

TABLE VI. Rabbits showing unusual reactions when inoculated intravenously with 0.01 mg. of human viruses.

i.v. = intravenous inoculation; s.c. = subcutaneous.

No. of Case.	Type of Case.	Culture used.	Dose mg.	Route	Post-mortem findings.
1796	Male, aged 17 mths. Tabes mesenterica; Tuberculous meningitis; generalised miliary tuberculosis.	3rd Sub.	0.01	i.v.	Dead 30 days. Blue beveran rabbit. Emaciated. Lungs, voluminous, confluent caseous lesions; caseous foci in tracheal glands. Few small white foci in both kidneys.
		4th Sub.	10	s.c.	Killed 68 days. Chronic lesion with caseous centre in left inguinal region & inguinal glands involved in lesion. Few caseous foci in right inguinal glands. Scanty caseous points in lungs.
S.20	Female, aged 11 mths. Tuberculous osteitis of humerus.	2nd Sub.	0.01	i.v.	Dead 35 days. Blue beveran rabbit. Slight loss in weight. Lungs not collapsed, caudal lobes on both sides almost entirely caseous, smaller lesions elsewhere, tracheal glands enlarged. Few small foci in kidneys.
		3rd Sub.	10	s.c.	Killed 68 days. Caseous lesion in left inguinal region. Scanty caseous foci in right inguinal glands and lungs.
1724	Female, aged 7 yrs. Caseous focus in lungs, slight cavitation, caseous thoracic glands. Caseous mesenteric glands in ileo-caecal angle. No miliary lesion and no meningitis.	1st Sub. (Bronchial glands).	0.01	i.v.	Killed 67 days. Small fibro-caseous lesions in lungs only.
		Eugonic 3rd Sub. (Mesenteric glands).	0.01	i.v.	Killed 37 days. Much emaciated. Generalised tuberculosis involving lungs, liver, spleen, kidneys, tracheal and axillary glands. Dysgonic culture isolated from kidney.
		Dysgonic 3rd Sub. (Rabbit kidney).	0.01	i.v.	Dead 34 days. Generalised miliary tuberculosis.
1876	Male, aged 2 yrs. 11 mths. Caseous mesenteric glands, with early calcification. Slight acute miliary tuberculosis, tuberculosis of cervical spine and cervical glands in relation; tuberculous meningitis.	2nd Sub.	0.01	i.v.	Killed 66 days. A few small (pea-sized) caseous foci & many smaller ones in lungs. In one tracheal gland a caseous focus. Few small whitish foci in kidneys.
2542	Male, aged 11 months. Caseous lung focus. Tuberculous thoracic glands - slight generalised sub-acute miliary tuberculosis; tuberculous meningitis.	3rd Sub.	0.01	i.v.	Killed 66 days. Two small (pea-sized) caseous foci in left caudal lobe and some small fibrotic lesions: scanty caseous points in tracheal glands and kidneys.
1942	Male, aged $8\frac{1}{2}$ years. Chronic pulmonary tuberculosis; chronic pleurisy; tuberculous thoracic glands; tuberculous meningitis.	3rd Sub.	0.01	i.v.	Killed 69 days. A few moderate-sized caseous foci in lungs and many widely separated smaller ones. Scanty foci in kidneys.
2185	Male, aged 9 years. Caseous lung focus, with fibrosis around. Chronic pleurisy, tuberculous bronchial glands; slight acute generalised miliary tuberculosis; tuberculous meningitis.	2nd Sub.	0.01	i.v.	Killed 67 days. Much emaciated. Emaciation began 4 weeks before death. Lungs voluminous with confluent caseous lesions. Generalised miliary tuberculosis. Dysgonic culture isolated from spleen.
		4th Sub.	10	s.c.	Killed 70 days. No loss in weight. Localised chronic ulcerated caseous lesion in left inguinal region and a few small caseous points in lungs.

kidneys (table VI, first part). In both cases the inoculation test was repeated by injecting 10 mg. subcutaneously into the inguinal region, and when the animals were killed 68 days later chronic caseous lesions were found at the site of inoculation, and while the related inguinal glands were involved in the lesions, those on the opposite side were the seat of scanty caseous points. The only other lesions were a few pin-head size caseous foci in the lungs.

In another case (1724 in table VI) 0.01 mg. of a culture obtained from mesenteric glands, and which showed definite eugonic characters only in the third sub-culture, caused the animal to fail rapidly after inoculation, and as death seemed imminent it was killed on the 37th day, when a widespread generalised tuberculosis was found. From the same case 0.01 mg. of a culture obtained from the tuberculous bronchial glands, and which showed definite eugonic characters in first sub-culture, was inoculated into an animal which was killed 67 days later when minimal lesions were found in the lungs and nothing elsewhere. In the human case (female, aged 7 years) from which these cultures were derived, there were two probable portals of entry, one abdominal and the other thoracic, but in addition there was some slight cavitation in the lung lesion, but no evidence of ulceration of the intestine such/

such as is usually seen in sputum infection in the child. It was surmised then that the discordant results in the animal test and the cultures raised from mesenteric gland were due to a mixed infection with human and bovine bacilli. This was proved by isolating from the kidney of the rabbit dead of generalised tuberculosis a typical dysgonic strain, which, on being inoculated in a dose of 0.01 mg. intravenously into another rabbit, killed the animal on the 34th day with generalised lesions. Further, on plating out a sub-culture of the one used for the original inoculation on glycerin egg medium two types of colonies resulted which on sub-culture on the various differential media gave in the one case a typical eugonic pigmented growth and in the other a dysgonic non-pigmented growth. It thus seemed likely that the abdominal lesion in the child was primarily due to an infection with a bovine type of bacillus, but superimposed on this there had been an infection with the human type from the thoracic lesion caused by the child swallowing the material coughed up from the early dissolution of the lung lesion.

Of the 51 remaining animals in this group killed 65 days or more after inoculation, all had minimal pulmonary lesions except 4 (table VI, second part). In 3 of these the lung lesions were of moderate severity, being composed of scanty small (pea-size) irregular caseous areas/

areas; in 2 cases the tracheal glands showed caseous points, while in all, the kidneys were the seat of scanty small caseous lesions, and there was no evidence of generalisation. The remaining animal (2185) out of these 4 had been inoculated from a typical eugonic culture obtained from bronchial glands and had remained in good health for about five weeks when it commenced suddenly to fail, and when killed on the 67th day a fairly extensive generalised tuberculosis was found with confluent caseous lesions in the lungs. On isolating the infecting organism from the animal's spleen a dysgonic type of growth resulted, but on plating out on glycerin egg a sub-culture of the original growth used for inoculation, all the colonies were of the same eugonic type and inoculation of 10 mg. subcutaneously of the same sub-culture as used for the plating produced in the animal, after 70 days, only a localised lesion with a few caseous points in the lungs. In this case the original animal may have contracted a bovine infection from one of the other animals inoculated with this type.

Bovine strains.

Results.

Of the 89 bovine strains isolated 87 were tested by animal experiment, 0.01 mg. of each culture being inoculated intravenously, one animal being used for each/

each case. The remaining 2 strains not tested by animal experiment were, as already noted, isolated from secondary lesions and corresponded in their cultural characters with those obtained from the primary foci. In 77 animals death occurred 44 days or less after inoculation, the average duration of life being 32 days, while other 8 died between the 45th and 60th days. In these 85 animals the lungs were riddled with very numerous small caseous lesions which, in some cases, had become confluent, producing larger caseous areas, while the other organs, lymphatic glands, and bone marrow were involved to varying degrees in an acute generalised miliary tuberculosis. From these observations it was evident that the strains of bacilli inoculated were of a high degree of virulence for the rabbit and corresponded to the bovine type. In the case of the 8 animals living 45 to 60 days (table VII) the lesions were slower in developing and causing death, either on account of the higher resistance of the individual rabbits or due to the dose inoculated being less than that calculated. As in these cases the cultural characters corresponded to the results of the animal test, there was no need for further investigation. It is of interest to note, however, that in 6 of the animals living 45 to 60 days the cultures displayed more luxuriant growth after the 3rd and 4th transfers when/

TABLE VII.

Rabbits living 45 days or more after intravenous inoculation with 0.01 mg. of bovine uultures.

TABLE VII. **Rabbits living 45 days or more after intravenous inoculation with 0.01 mg. of bovine cultures.**

i.v. = intravenous inoculation; s.c. = subcutaneous.

No. of Case	Type of Case.	Culture used.	Dose mg.	Route	Post-mortem findings.
2858	Male, aged 6 yrs. Caseous mesenteric glands. Child dead of rheumatic carditis.	2nd Sub.	0.01	i.v.	Dead 45 days. Emaciated. Several large confluent caseous areas in lungs; caseous points in tracheal & axillary glands; numerous moderate-sized caseous foci in kidneys; spleen moderately enlarged & numerous caseous foci; scanty tubercles in liver; a few tubercles in rib marrow.
1542	Male, aged 1½ years. Tuberculous ulceration in small and large bowel; tuberculous peritonitis, caseous mesenteric glands; slight miliary tuberculosis.	3rd Sub.	0.01	i.v.	Dead 45 days. Slight emaciation. Large confluent tubercles showing caseation in lungs; bronchial nodes caseous; kidneys & spleen show moderate number of large caseous tubercles, small foci in liver and rib marrow.
S.28	Female, aged 11 yrs. Tuberculosis of right ankle.	3rd Sub.	0.01	i.v.	Dead 53 days. Slight loss in weight. Lungs not collapsed & many confluent caseous tubercles. Spleen enlarged, many moderate-sized tubercles. Kidneys, many large tubercles. Liver, a few foci. Bronchial and coeliac nodes slightly caseous. Peritoneum, tubercles in parietal & visceral layers
2527	Male, aged 3 yrs. 3 mths. Tuberculous ulceration of small intestine, caseous mesenteric glands.	2nd Sub.	0.01	i.v.	Dead 53 days. Emaciated. Lungs riddled with confluent caseous tubercles. Kidneys, numerous tubercles. Liver and spleen negative. Axillary, inguinal & bronchial nodes show small tubercles. A few pin-head size tubercles in peritoneal aspect of diaphragm.
2388	Female, aged 6 yrs. 4 mths. Dead of rheumatic carditis; tuberculous mesenteric glands.	3rd Sub.	0.01	i.v.	Dead 54 days. Slight emaciation. Lungs voluminous and riddled with confluent tubercles with caseous centres. Bronchial and coeliac nodes, caseous. Spleen enlarged and studded with medium-sized tubercles. Kidneys, moderate number of medium-sized tubercles. Liver, very many small tubercles.
2072	Male, aged 8 yrs. Dead of acute ileocolitis. Tuberculous mesenteric glands.	3rd Sub.	0.01	i.v.	Dead 57 days. Marked emaciation. Lungs riddled with confluent caseous tubercles. Bronchial, axillary, coeliac nodes, small caseous tubercles. Spleen much enlarged and studded with caseous tubercles. A few foci in liver. Many moderate-sized tubercles in kidneys.
S.24	Female, aged 6 yrs. Tuberculous disease of right fibula.	3rd Sub.	0.01	i.v.	Dead 59 days. Slight emaciation. Lungs, numerous large confluent caseous tubercles. Tubercles in pericardium and pleurae. Spleen, greatly enlarged & numerous caseous tubercles. Kidneys, numerous tubercles. Liver, scanty small tubercles. Bronchial, coeliac & retro-peritoneal lymph nodes enlarged and caseous.
2405	Female, aged 4 yrs. Tuberculosis of cervical and upper mediastinal glands; slight sub-acute miliary tuberculosis; tuberculous meningitis.	2nd Sub.	0.01	i.v.	Dead 60 days. Slightly emaciated. Lungs solid with large confluent caseous tubercles. Bronchial, axillary & retro-peritoneal nodes enlarged and caseous. Spleen moderately enlarged & studded with caseous foci. Kidneys, numerous large caseous tubercles. Liver, scanty caseous points. Tubercles in rib marrow.

when the growth on glycerin egg equalled that on ordinary egg, though that on glycerin potato still remained poor. None of these 6 strains had produced definite pigment. With the remaining 2 strains (S.57, S.18, table VIII) which had shown cultural characters similar to these just described the position was somewhat different and the results obtained will be described more fully. Both strains were isolated through guinea-pigs from cervical glands of children, one aged 8 years 3 months, the other 11 years 4 months. On inoculation intravenously of 0.01 mg. of culture from 3rd transfers on ordinary egg the animals were still alive 60 and 62 days afterwards. Both were killed and at post-mortem the lungs were found to be only moderately studded with small caseous lesions. The miliary lesions in the other organs and tissues were not so acute or extensive as those produced by the other bovine strains investigated, the number and size of the tuberculous foci in the spleen and kidneys being less. No lesions were noted in the liver, and in the lymphatic system small caseous foci were noted only in the tracheal glands and in one case also in the coeliac glands. Thus the reaction produced corresponded with that given by some of the human strains. Both strains were re-isolated from the lesions in the kidneys. While awaiting the results of the cultural tests other two rabbits were inoculated/

TABLE VIII.

Rabbits living 45 days or more after intravenous inoculation with 0.01 mg. of bovine cultures and showing unusual reactions.

TABLE VIII. Rabbits living 45 days or more after intravenous inoculation with 0.01 mg. of bovine cultures and showing unusual reactions.

i.v. = intravenous inoculation; s.c. = subcutaneous.

No. of Case.	Type of Case.	Culture used.	Dose mg.	Route	Post-mortem findings.
S.57	Female, aged 8 yrs. 3 mths. Tuberculous cervical adenitis. (operation).	3rd Sub.	0.01	i.v.	Killed 60 days. Gain in weight. Lungs, moderate number of small (lentil-sized) caseous lesions. Small caseous points in tracheal & coeliac nodes. Spleen, slight enlargement & a few small caseous foci. Kidneys, moderate number of lentil-sized caseous foci. Liver, nil.
		4th Sub.	10	s.c.	Dead 75 days. Local caseous lesion at site of inoculation, ulcerated. Right and left inguinal, iliac & retro peritoneal nodes caseous. Lungs studded with a fair number of small caseous tubercles. Spleen slightly enlarged & a few moderate-sized caseous foci. Kidneys, a few small & large caseous tubercles (one 0.5 cm.) Liver, a few caseous points.
		3rd Sub. (re-isolated from kidney of 1st rabbit)	0.01	i.v.	Dead 55 days. Slight emaciation. Lungs riddled with small caseous lesions. Bronchial & axillary nodes, a few tubercles. Spleen, slightly enlarged & numerous caseous points. Liver, a few tubercles. Kidneys, numerous caseous foci.
S.18	Male, aged 11 yrs. 4 mths. Tuberculous cervical adenitis. (operation).	3rd Sub.	0.01	i.v.	Killed 62 days. Slight emaciation. Lungs, moderate number of small caseous lesions; in left caudal lobe a large confluent caseous area Tracheal nodes enlarged and slightly caseous. Moderate enlargement of spleen and a few pea-sized caseous lesions. Kidneys, scanty (9) moderate-sized caseous foci. Liver, nil.
		4th Sub.	10	s.c.	Dead 58 days. Large local caseous lesion at site of inoculation, ulcerated. Both inguinal, and iliac nodes enlarged and caseous. Lungs, voluminous, a few confluent caseous tubercles and some smaller ones. Spleen slightly enlarged and some small caseous foci. Kidneys, a few moderate-sized caseous lesions. Liver, scanty small foci.
		3rd Sub. (re-isolated from kidney of 1st rabbit)	0.01	i.v.	Dead 62 days. Slight emaciation. Lungs, several confluent caseous areas & many smaller caseous foci. Bronchial & coeliac nodes caseous. Spleen, enlarged & studded with many caseous foci. Kidneys, many caseous tubercles. Liver, a few pin-head size tubercles. Diaphragmatic peritoneum studded with small tubercles

inoculated subcutaneously in the inguinal region with 10 mg. of sub-cultures of those used for the original inoculation. One of these animals died 58 days later and the other 75, both with slight generalised miliary tuberculosis and ulcerated local caseous lesions; the inguinal and iliac glands on both sides were also extensively caseous. The cultural characters of the strains re-isolated from the first series of animals remained much the same as in the original cultures derived from human material, though the strain which originally showed the luxuriant growth on glycerin egg after the 4th sub-culture now showed that in the sub-culture on glycerin egg made from the 3rd transfer on ordinary media. The growth of both strains on glycerin potato, however, still remained poor. On inoculation of rabbits with 0.01 mg. intravenously of the third sub-cultures of the re-isolated strains one animal died 55 days later and the other 62, both showing a more acute generalisation than in the animals similarly inoculated from the original cultures, though the lesions produced were still not as severe as those produced by the other bovine strains. On the other hand they were more widespread than the acute lesions produced by certain of the human strains. Accordingly, it was concluded that the two strains, while of bovine type, fell below the usual standard of virulence for the rabbit/

rabbit, though in this respect they were not as low as the bovine strains obtained from the lupus cases reported by the Royal Commission (1911 (6))

(4) <u>The correlation of cultural characters and animal tests</u>.

The pathological lesions produced in the inoculated rabbits were chiefly relied on for the diagnosis of type. Most of the typical eugonic cultures produced either slight localised or no lesions in the rabbit, while severe generalised lesions were produced by most of the dysgonic, yet from the preceding notes it will be recognised that this did not invariably occur, the results of a few of the animal tests given by the two types of bacilli not being strictly comparable with the cultural results. Apart from this, where there was any marked disagreement both sets of experiment were subject to re-investigation, and in this way one case of mixed infection in the child and another of probable crossed infection in the animal were discovered. In addition this correlation acted as a check on the increased susceptibility to infection with human strains on the part of the individual animals used. In the case of the less typical cultures, it was found that out of the 12 more luxuriantly growing bovine strains isolated the animals inoculated with these had survived longer than usual in 8 cases/

cases, in 2 of which the lesions were less acute and less widespread than those produced by the more typical dysgonic strains. This finding corresponds with what Park and Krumwiede (1910 (7)) have noted when they said "roughly the virulence is inversely proportional to the amount of growth," and while we found this to be true to a certain extent with bovine strains, yet on the other hand the less eugonic human strains showed no increase in virulence for the rabbit, though a few of the typical eugonic were more virulent than usual for the individual animals used.

The correlation of the two sets of results (cultural and animal) however gave us sufficient data for the definite diagnosis of the types of infecting bacillus in the various lesions studied.

SECTION III.

Autopsy Cases.

SECTION III.

Autopsy Cases.

The incidence of tuberculous lesions according to age.

Having now got the organisms typed, it is opportune at this point to discuss the various lesions from which an attempt was made to isolate the infecting virus. The subjects, composing the 1,300 consecutive autopsies studied for evidence of tuberculous lesions, consisted of children whose ages ranged from a few days old to between 12 and 13 years, 1,033 (79.5 per cent.) being under 2 years. Of these 1,300 subjects, 216 (16.6 per cent.) showed tuberculous lesions. The incidence of these according to age groups is shown in table IX, from which it is evident that the greatest number of cases, 75, were observed in the first year of life, while the second year came next with 53. Findlay (1926) has also observed this on the clinical side. By expressing the number of cases with tuberculous lesions in each age group as a percentage of the total number of post-mortems in the group it was found that in the first 3 months the percentage was very low, being only 1.2 per cent. of 410 cases. After that there was a progressive increase throughout the various quarters of the first year, the two halves of the second year, up to 45/

TABLE IX.

Incidence of tuberculous lesions according to age groups in 1,300 consecutive autopsies.

Age group.	Total autopsies.		Tuberculous Cases.			
			Number		Percentage of total P.M's in each age group	
0 to 3 mths.	410		5		1.2	
3+to 6 "	212	856 in 1st year.	22	75	10.4	8.8
6+to 9 "	143		27		18.9	
9+to 12 "	91		21		23.1	
12+to 18 "	118	177 in 2nd year.	31	53	26.3	29.9
18+to 24 "	59		22		37.3	
2+to 3 yrs.		60		27		45.0
3+to 4 "	34		12		35.3	
4+to 5 "	46	97 from 3+ to 6 years.	14	34	30.4	35.1
5+to 6 "	17		8		47.1	
6+to 7 "	34		9		26.5	
7+to 8 "	28	76 from 6+ to 9 years.	2	18	7.1	23.7
8+to 9 "	14		7		50.0	
9+to 10 "	11		1		9.1	
10+to 11 "	12	34 from 9+ to 12+ years.	5	9	4.2	26.5
11+to 12+ "	11		3		27.3	
Totals	1,300		216		16.6	

45 per cent. in the 2 to 3 year age group. This was also evident if the percentages in yearly age groups were taken instead of these shorter periods, the percentages for the first three years being 8.8 of 856 cases in the first, 29.9 of 177 cases in the second, and 45.0 of 60 cases in the third. There was, therefore, a steady increase in the frequency of tuberculous lesions from the early months of infancy up to between the second and third years. This has also been noted by Simmonds, Schwer and Boltz (1873-1889), Kuss (1898), Hamburger and Sluka (1905) and Wollstein (1909). A comparison of our percentages with those of Wollstein is given in the following table. It is interesting from the point of view of their fairly close similarity, and from the fact that they were in children only up to 3 years of age, with whom the selective factors governing the admission of such cases to general hospitals, as already noted in the introduction, probably played little part.

	0-3 months	3-6 months	6-9 months	9-12 months	12-18 months	18-24 months	2-3 years
Wollstein	1.8	11	16	23	34	44	32
Blacklock	1.2	10.4	18.9	23.1	26.3	37.3	45

As to the cause of this increase all continental authors are agreed that it is due to the fact that during the second year children begin to move about for themselves, and, particularly in the crawling stage, get their hands contaminated with dust and dirt infected with the tubercle bacilli (<u>l'infection de touche à tout</u> of French authors, and <u>Schmutz-infektion</u> of the German). In other words, according to these authors, before the second year of life the tuberculous infection is conveyed directly to the child by one suffering from "open" tuberculosis, whereas from the second year onward in addition to this children may infect themselves by conveying the bacilli picked up from the ground, floors, furniture, etc., with their hands to the mouth. In fact, Dieudonné (1901), investigating the dirt from the hands of children aged 9 months to 2½ years for the presence of tubercle bacilli, recovered these by inoculation of guinea-pigs in 2 out of 15 cases. One of the children was aged 15, and the other 18 months. In support of this, as we shall point out later, it was at this period that we found the greatest number of abdominal tuberculous lesions infected with the human type of bacillus. In addition to this assumption on the part of continental authors that the increase is due to the child being brought more into contact with sources of infection after the first year of life, we must also consider the likelihood of in-fection/

infection from milk. It is in infancy and early childhood that large amounts of milk are taken by the child. During the first year of life the milk is often boiled in the preparation of infant foods, but after that period this is not so, and, as we shall show later, the greatest incidence of the bovine type of infection was noted in our series of cases between the first and second years.

After the third year the numbers of cases with tuberculous lesions and total number of autopsies became too small to be of value for comparing the incidence of tuberculous lesions in any one year with that in another. Accordingly, it is not possible for us to arrive at definite conclusions regarding the yearly incidence of tuberculous lesions in children over 3 years in this investigation, as the figures can be manipulated to show discordant results. It is of interest, however, to note the incidence of tuberculous lesions in these later years of childhood by taking all the cases occurring in periods of 3 years from the third year onwards. When this was done a fall was noted in the percentage from 45.0 between the second and third years to 35.1 between the third and sixth, and from that to 23.7 between the sixth and ninth, then a slight rise to 26.5 between the ninth and twelfth.

In view of the general reduction in the morbidity and mortality rates of tuberculous disease not alone in this/

this country, but also abroad, it is interesting to compare our results with those of Shennan (1914). This worker conducted a similar investigation to ours at the Royal Hospital for Sick Children, Edinburgh, during the years 1886 to 1902, and 1910 to 1913, in 1,277 children whose ages were given, and in whom 497 cases with tuberculous lesions were found. In the following table his percentages of cases showing tuberculous lesions to total number of post-mortems in each age group are compared with ours.

	0-1 year.	1-3 years	3-5 years	5-14 (S)* 12 (B)	Total
Shennan (1886-1902 & 1910-1913)	17.6	43.5	49.7	53.1	38.9
Blacklock (1924-1930)	8.8	33·8	32.5	27.6	16.6

* Shennan's cases extended to 14 years, and our own to between 12 and 13 years.

How far a comparison of these results can be taken as showing a reduction in the incidence of tuberculous lesions throughout all the age groups is difficult to say, for, as we have already noted, numerous selective factors must be taken into account. These figures are, however, suggestive/

suggestive that there has been a reduction in the incidence of tuberculous lesions throughout the whole of infancy and childhood.

By what other means could we arrive at the incidence of tuberculous infection in the child? The cutaneous (von Pirquet) and intra-cutaneous (Mantoux) reactions, while helpful, often fail in the case of the child suffering from advanced tuberculous disease. It is interesting, however, to note that Hamburger and Monti (1909), using the cuti-reaction, and where this was negative the intra-cutaneous, in a series of children in Vienna found that there was a steady increase in the incidence of positive reactions from 1 per cent. in the first year of life to 94 per cent. for children between the ages of 11 and 14 years. Thus, while the incidence of tuberculous lesions recognised at post-mortem and positive tuberculin tests showed an increase up to between the second and third years, the former decreased after that period, while the latter went on increasing. What, then, is the explanation? It seems to us that this difference is due to the tuberculous disease, after the third year of life, becoming more localised chiefly in lymphatic glands, which, though diseased, retain the infection. Further, as we have noted in the present investigation, there was an increased tendency to the arrest of the disease after this period, as shown by the fibrosis of the lesions, and often actual calcification.

TABLE X. Incidence of tuberculous lesions according to sex and age group in 1,300 post-mortems.

Age group.	Boys. Total Autopsies		Boys. Tuberculous Cases. Number		Boys. Tuberculous Cases. Percentage		Girls. Total Autopsies		Girls. Tuberculous Cases. Number		Girls. Tuberculous Cases. Percentage	
0 to 3 mths.	266	525 in 1st year	4	46	1.5	8.8	144	331 in 1st year	1	29	0.7	8.8
3+ to 6 "	130		17		13.1		82		5		6.1	
6+ to 9 "	79		12		15.2		64		15		23.4	
9+ to 12 "	50		13		26.0		41		8		19.5	
12+ to 18 "	70	104	18		25.7		48	73	13		27.1	

TABLE X.

TABLE X. Incidence of tuberculous lesions according to sex and age group in 1,300 post-mortems.

Age group.	Boys. Total Autopsies		Boys. Tuberculous Cases. Number		Boys. Tuberculous Cases. Percentage		Girls. Total Autopsies		Girls. Tuberculous Cases. Number		Girls. Tuberculous Cases. Percentage	
0 to 3 mths.	266		4		1.5		144		1		0.7	
3+ to 6 "	130	525 in 1st year	17	46	13.1	8.8	82	331 in 1st year	5	29	6.1	8.8
6+ to 9 "	79		12		15.2		64		15		23.4	
9+ to 12 "	50		13		26.0		41		8		19.5	
12+ to 18 "	70	104 in 2nd year	18	30	25.7	28.8	48	73 in 2nd year	13	23	27.1	31.5
18+ to 24 "	34		12		35.3		25		10		40.0	
2+ to 3 yrs.		29		15		51.7		31		12		38.7
3+ to 4 "	20	50 from 3+ to 6 years.	4		20.0		14	47 from 3+ to 6 years.	8		57.1	
4+ to 5 "	19		2	11	10.5	22	27		12	23	44.4	48.9
5+ to 6 "	11		5		45.5		6		3		50.0	
6+ to 7 "	18	43 from 6+ to 9 years.	4		22.2		16	33 from 6+ to 9 years	5		31.3	
7+ to 8 "	17		1	10	5.9	23.3	11		1	8	9.1	24.2
8+ to 9 "	8		5		62.5		6		2		33.3	
9+ to 10 yrs.	9	21 from 9+ to 12+ years.	1		11.1		2	13 from 9+ to 12+ years.	-		-	
10+ to 11 "	5		1	4	20.0	19.0	7		4	5	57.1	38.5
11+ to 12+ "	7		2		28.6		4		1		25.0	
Totals	772		116		15.0		528		100		18.9	

The incidence of tuberculous lesions according to sex.

In table X the sex incidence of the various lesions is noted. Of 772 male children examined 116 (15.0 per cent.) showed tuberculous lesions, while similar findings were noted in 100 (18.9 per cent.) of 528 female children. Up to the end of the second year there was little difference in either sex in the percentage of tuberculous cases to the number of post-mortems when only yearly age groups were studied, though this was not absolutely borne out on comparing the percentages in the smaller age-groups composing these periods. In the second to the third year group the percentage in boys was higher than girls, but after this, as in the case of the total lesions, the numbers became too small for comparison by periods of one year. When all the cases occurring in periods of 3 years were taken from the end of the third year the incidence of tuberculous lesions was found to be commoner in girls from the 3 to 6 year age group onwards, though it is to be noted that on account of the small numbers this was not absolutely so when the sex incidence in the one year periods was compared.

The above results have been expressed graphically in table XI, from which it is seen that the percentage of cases with tuberculous lesions to the total post-mortems rises/

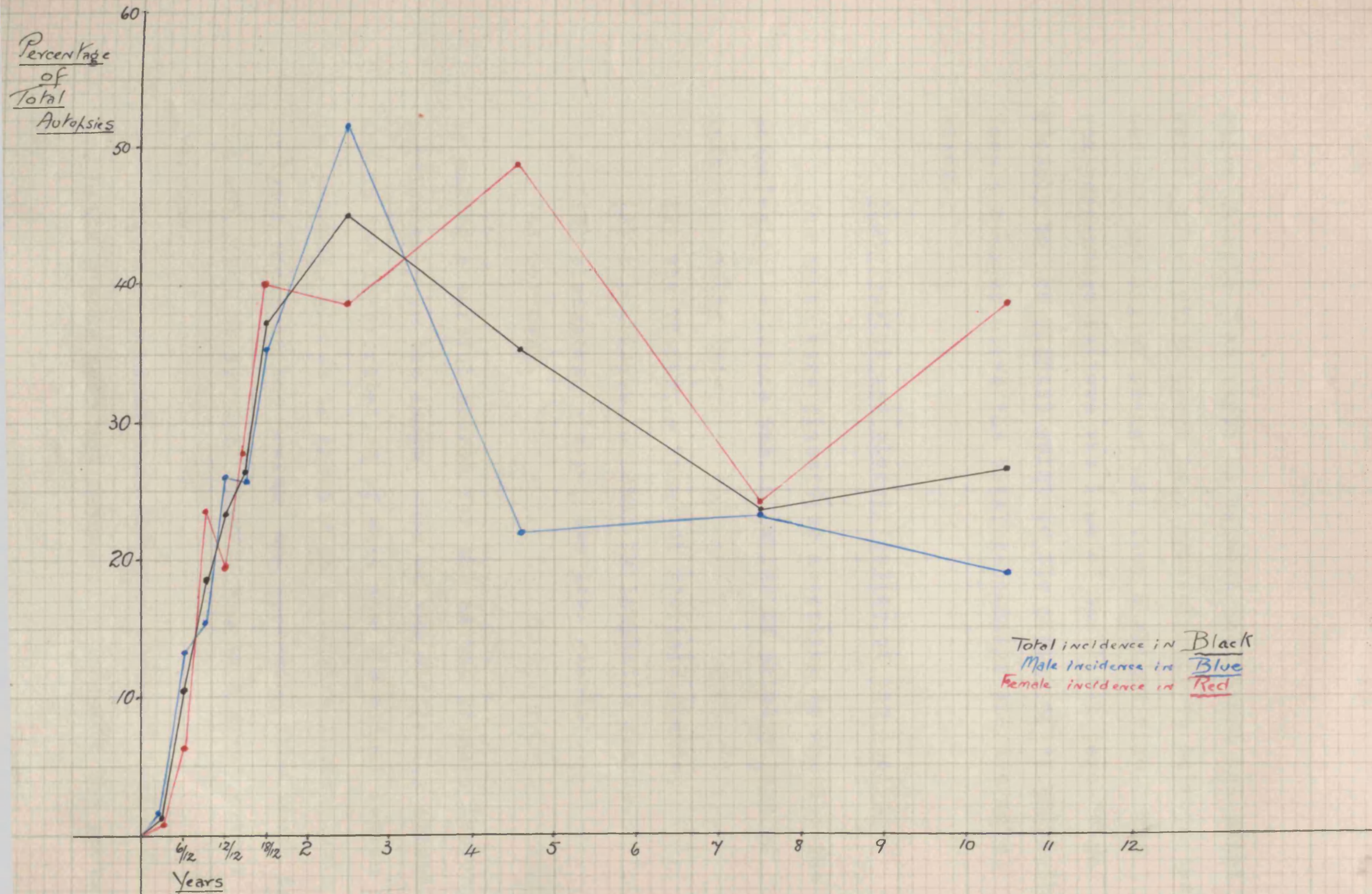

Table XI. Graphic representation of incidence of tuberculous lesions according to age groups and sex in 1,300 autopsies.

rises sharply from birth to between the second and third years, after which there is a gradual fall to between the seventh and eighth years, and then a slight rise. The sex percentage follows the line of the total percentage closely to the highest point in the graph, after which the female remains above the total percentage and the male below.

Distribution and classification of the lesions.

The cases were classified according to whether the pathological evidence was in favour of primary site of infection being in:-

(1) the thorax, - lungs or thoracic glands;

(2) the abdomen, - bowel or lymphatic glands;

(3) elsewhere, - e.g., cervical glands;

(4) unknown.

By making a careful search for the actual primary lesion, particularly in the lungs, much of the difficulty attendant on the interpretation of what was the oldest lesion in lymphatic glands in cases with extensive tuberculosis in lymph nodes in the thorax, and spreading from there to the abdominal or cervical glands, was to a great extent overcome.

It is recognised that the most advanced lesion in lymphatic glands does not necessarily indicate the portal of/

of entry of the tuberculous infection, for the contrary has been shown experimentally. Krause (1919) inoculated guinea-pigs subcutaneously in the groin, with a strain of tubercle bacilli of low virulence for that animal, and found that often the bronchial glands showed more marked morbid change than those in relation to the site of inoculation. In fact, he stated that if the site of inoculation had been unknown, a conclusion might have been reached that the portal of infection was in the respiratory system, though no lesion was found in the lungs of the guinea-pigs he used. Later (1920), he obtained almost similar results with a human strain of average virulence. We have also noted similar findings in mice inoculated intraperitoneally, and in which after an interval the lungs and bronchial glands showed advanced tuberculous disease, while the peritoneal lesion had almost entirely cleared up. Krause's observation can be criticised on the grounds that in the guinea-pig, even in advanced stages of tuberculous infection, the lungs, as compared with other organs, remain relatively free of lesions. This is due, as noted by Hashiba (1917) and Krause (1920), to the lymphatics of the lung in the guinea-pig being very wide, and also to the relative absence of lymphoid tissue in these organs. Further, the doses used in the experiments quoted above were larger than those occurring in the natural infection in/

in man, and also the guinea-pig is more easily infected. In the case of the mouse, tissue reactions are uncertain, and we have found that only some strains of human and bovine bacilli will produce definite tuberculous lesions.

These experimental findings are contrary to the laws of Cohnheim and Cornet. The former law states that where bacilli have passed through lymphatic glands and caused tuberculous disease elsewhere, evidence of infection will also be found in the glands in relation to the portal of entry. The latter law holds that in tuberculosis a primary lesion is produced at the point where the bacilli invade the body, in addition to a tuberculous lesion in the related lymph nodes. These laws do not, however, allow for the now well-established fact that tubercle bacilli can remain latent for a short time at least in the lymphatic glands, as already described, or that tubercle bacilli may be taken up by phagocytic cells and passed through a mucous surface without producing any morbid change therein. Also, the virus may pass through successive groups of glands without causing any lesions, coming to rest at a distance from the portal of entry, and there producing a lesion when the resistance of the tissue is lowered either through local or general causes. Further discussion on this question of the portal of entry is reserved till later, when the results of our work have been given.

(1) Cases in which the primary site of the infection was considered to be in the thorax.

It has never been seriously debated that in a case where the only site of tuberculous disease in the human body was in the mesenteric glands the primary site of the infection was other than intestinal, even although no evidence of ulceration was found in the mucosa of the bowel. The significance of tuberculous tracheo-bronchial glands is different, however, in that they can become the seat of tuberculous disease in cases of aerogenous or hematogenous infection of the lungs, or by the spread through lymphatic channels of a tuberculous infection - latent or otherwise - from other sites. Were it possible to recognise some lesion in the lung which could be regarded as primary, and undoubtedly due to aerogenic infection, much of the difficulty with regard to the significance of tuberculous tracheo-bronchial glands would be removed. A lesion of this nature, however, would require to be different both in the histological and anatomical appearances from those produced by secondary hematogenous, lymphogenous or intracanalicular infection Further, it would need to be capable of being reproduced experimentally. Such lesions have been described, and have been termed the primary lung focus (chancre d'inoculation of the French authors, primare Lungenherd of the German, and

focal/

focal tuberculosis of the American). This focus, together with related diseased lymph nodes, is often referred to as the primary complex (Ranke, 1928).

Anatomical considerations.

Before proceeding to a description of our own observations and those of others regarding the primary focus in the lung, it might be opportune here to review very briefly the lymphatic apparatus of the lung as far as this has a bearing on the path of infection in tuberculous disease. By doing this, confusion may be avoided by defining clearly the various anatomical terms used. The lymphatics of the lungs have been divided into two sets, a deep in the substance of the lung, and a superficial plexus in the pleura. As demonstrated by Miller (1919) these two sets of lymphatics are practically independent, only scanty anastomoses existing between them, consisting of short, valved lymphatics which accompany the small tributaries of the pulmonary vein that take origin in the pleura. The valves in these junctional lymphatics point towards the pleura, thus normally preventing any flow of lymph from the pleural plexus into the deep lymphatics of the lung. A single lymphatic plexus is present in the walls of the smaller bronchioles, but ends in the region where these terminate, that is, distal to/

to the origin of the ductuli alveolares. Here the plexus divides into three small vessels, two of which join the lymphatics around the branches of the pulmonary vein, while the third joins the lymphatics around the pulmonary artery (fig.1). Thus there are probably no lymphatics in the walls of the atria or air vesicles. The superficial and deep lymphatics drain into the broncho-pulmonary glands at the hilum.

Small masses of lymphoid tissue are found in the neighbourhood of the bifurcations of the smaller bronchi and bronchioles, at the distal ends of the ductuli alveolares, along the course of the pulmonary artery and pulmonary vein, and a small focus of lymphoid tissue is also found in the pleura where the branch of the pulmonary vein arises from that structure. It is in these small collections of lymphoid cells that the lymphatic vessels take origin (Miller, 1911). The lymphatic glands in relation to the lungs, bronchi and trachea are the tracheo-bronchial, (Sukiennikow, 1903), though often described as the mediastinal and bronchial in medical literature. They are divided into 4 sub-groups (fig.2) as follows:-

(a) <u>Broncho-pulmonary glands</u> which are very small, and consist of an intra- and extra-pulmonary group. The former are very small nodes, and are found along the bronchi up to divisions of the fourth order. The extra/

extra-pulmonary nodes which are also small are situated in close apposition to, and between the branches of the main bronchi at the hilum of the lung.

(b) <u>Superior tracheo-bronchial glands</u>, consisting of 3 or 4 small nodes on each side and situated on the upper aspect of the main bronchus in the angle formed by the trachea and the origin of the bronchus to the upper lobe.

(c) <u>Inferior tracheo-bronchial glands</u>, consisting of 4 or more large glands on each side and situated below the bifurcation of the trachea in relation to the bronchi on either side.

(d) <u>Para-tracheal glands</u>, found along either side of the trachea.

The tracheo-bronchial lymph nodes are strictly regional glands, and, as shown by Most (1908 (1)) and Beitzke (1908), have no direct connection with the cervical or abdominal lymph nodes. Ghon (1916) has shown from his studies on tuberculosis in the human subject, and Scott and Beattie (1928) from similar studies as well as purely anatomical ones in the case of the primates, that the superior tracheo-bronchial glands receive lymph for the most part from the upper lobe on the corresponding side, while/

while the inferior group receives lymph from the lower lobes on the same side, and, in the case of the right, also from the middle lobe. While this is the rule, yet it is not absolute, as Most (1908 (2)) showed that the pleural lymphatics in the interlobar fissures unite in the deep parts of these fissures to form common channels of discharge from the pleural surface of neighbouring lobes. The inferior tracheo-bronchial glands drain into the superior group of glands on the same side, while the superior and inferior sets on one side have also communi-cations with the corresponding groups on the other. This intercommunication between corresponding groups of glands is particularly well-developed in the case of the inferior glands. No intercommunication with the glands on the other side is found, however, in the case of the broncho-pulmonary glands, though on the same side they are intimately con-nected with one another. Both sets of superior tracheo-bronchial drain into the corresponding thoracic para-tracheal glands situated along the right and left sides of the trachea. Intercommunicating lymphatics also exist between the para-tracheal glands on one side and those on the other. The efferents from these glands join with those of the superior mediastinal and internal mammary glands, forming on each side a common broncho-mediastinal trunk which drains into the thoracic duct or the right lymphatic/

lymphatic duct. On the left side, however, the efferents from the tracheo-bronchial glands may end directly in great veins or thoracic duct.

The important anatomical points which have a bearing on tuberculous infection are as follows:-

(a) The broncho-pulmonary glands at the hilum of each lung are related to the lung on one side only. The area drained by the superior and inferior tracheo-bronchial glands is also fairly sharply demarcated. If there is a tuberculous focus in the lung the glands related to the area in which the focus is present should thus become involved first, and as time goes on and there is spread to other glands they should show more advanced disease than other lymph nodes.

(b) Actual lymph nodes occur within the lung substance through which the path of infection may sometimes be traced to the glands at the hilum. Further, these small nodes may sometimes be the seat of the primary lesion.

(c) Small foci of lymphatic tissue are present along the bronchi and bronchioles right up to the origin of the ductuli alveolares. These foci, like the smaller lymph nodes, may also be the site of a primary tuberculous lesion.

(d) The small communicating channel between the deep and superficial (pleural) sets of lymphatics can be used when the deep lymphatics become obstructed by tuberculous disease. Thus infection may readily spread to the pleura from a tuberculous focus in the lung.

(e) From the termination of the bronchioles there are four possible channels of drainage to the hilum:- firstly through the single plexus around the bronchioles, and then through the double plexus around the bronchi; secondly by the lymphatics accompanying the pulmonary artery; thirdly by those accompanying the vein, and fourthly, particularly if the other channels are blocked, by the anastomosès between the deep and superficial systems, and then through the pleural lymphatics. Accordingly, it is likely that any tuberculous infection in the region of the termination of the bronchioles would be readily transported by lymphatics to the glands around the hilum.

Observations on the pathology of the primary lesion in the lung by other authors.

The fact that the tracheo-bronchial glands were never the seat of tuberculous disease without a corresponding lesion being present in the lung in a series of 145 cases of tuberculosis in children aged 1 to 7 years which Parrot/

Parrot (1876) investigated led him to regard these glands "as the mirrors of the lungs." This has been termed "la loi des adenopathies similaires" or Parrot's law by French authors. Parrot considered that the lesion in the lungs in these cases was primary. Later, Kuss (1898), E. Albrecht (1907), H. Albrecht (1909), Gohn and Roman (1913), Gohn (1916), Opie (1917), Canti (1919), Schürmann (1926), and Ranke (1928) made similar observations to Parrot. These authors found that the focus in the lung causing the diseased glands was generally single, and on the few occasions in which they were multiple they were often related to one another. For the most part they varied in size from that of a pinhead to a hazel-nut, though larger ones were occasionally met with. These foci were generally fairly sharply demarcated from the surrounding lung tissue which in the earlier stages of the lesion showed no gross evidence of atelectasis or emphysema such as is usually seen around acute inflammatory lesions. The foci were usually found in the caseous stage, though often further retrogressive changes were present, e.g., encapsulation, with fibrous tissue, calcification or cavitation. Sometimes fresh miliary tubercles due to lymphatic spread from the focus were found in the lung tissue around. H. Albrecht noted that in the centre of such a lesion a slit could often be made/

made out, corresponding to the remains of a caseous bronchiole. Occasionally instead of being single the primary lesion consisted of an aggregation of small, dense tubercles. When small, the primary lung focus was difficult to find, and often long search was necessary. Although the focus might be minute the associated lymph nodes were much enlarged and caseous, this being due to the greater degree of inflammatory hyperplasia possible in lymphatic glands as compared with other tissues. It was only on rare occasions, if at all, that these various authors failed to find the primary focus which was occasionally situated deep in the lung substance, though more often immediately under the pleural surface. In some of the cases the pleura in immediate relation to the focus showed evidence of inflammatory change, e.g., adhesions, but these were rarely so strong that they could not be fairly easily broken through. As regards their situation in the various lobes, Kuss found them most frequently in the lower lobes, and Gohn and Schürmann in the right upper lobe.

The histological appearance of such a lesion in the earliest stage, as shown by Zarfl (1913) in a child aged 24 days, was that of a localised patch of typical tuberculous broncho-pneumonia. The alveolar walls were swollen and the air spaces filled with fibrino-cellular exudate/

exudate in which were abundant tubercle bacilli, but no endothelioid or giant cells. Gohn and Roman described a slightly later stage in a boy aged 5½ months. In their case, some caseous change had taken place in the centre of the fibrino-cellular exudate, but even at this stage these authors failed to find any tubercle follicles or giant cells, although there were abundant tubercle bacilli and no other organisms. Gohn and Pototschnig (1918) have also described a very early primary focus in the right middle lobe in a child aged 35 days. This case is of much interest as the glands in relation to the small primary pneumonic lesion which showed early breaking down were the seat of foci of endothelioid cells, but no giant cells, and in addition there were signs of early necrosis. In both focus and glands abundant tubercle bacilli were found. In all of these early cases the primary lung lesions showed no relation to vessels, and the authors concluded, partly on this account and partly from the histological appearances, that the foci were due to inhalation of the infecting organism. In the later stages the caseous process extended and involved all the intra-alveolar exudate, in addition to the walls of the air vesicles where the elastic fibres remained intact for some time; hence the pattern of the air vesicles and bronchioles in such a focus could still be demonstrated when/

when stained by Weigert's elastic tissue stain. This observation was of importance, as we shall discuss later. About this time, secondary typical miliary tubercles began to appear around the focus, being formed by lymphatic spread of the infection from the original focus, and from these in some cases was partly formed at a still later stage the enveloping fibrous capsule. This was the usual stage in which such foci were found in the lung. In fact, the early tendency to encapsulation and repair was peculiar to the primary focus, and served as a means of distinguishing it from other lesions where the tuberculous disease was progressive, with the formation of other lesions, e.g., tuberculous broncho-pneumonia and miliary tubercles. Still further stages have been described in which lime salts were deposited in the lesions which in some cases became petrified, while in a few cases there was true bone formation (Puhl, 1922). Even in the healing stage the air vesicles surrounding such a lesion showed little secondary involvement, and in only some cases was a slight degree of interstitial fibrosis noted. Accordingly, the healed focus was sharply marked off from the surrounding lung tissue. In the cases where healing did not take place there was a rapid extension of the tuberculous broncho-pneumonic process. This was more often seen in the infant, as, with advancing age, these various authors/

authors noted that there was an increasing tendency towards chronicity on the part of these primary lesions. Foci corresponding to those in the human subject have been experimentally reproduced in animals by Gardner (1922), and also by Pagel (1926).

From this description it will be seen that these foci were primarily of an exudative nature. If they were productive, then the pattern of the air vesicles as shown by the elastic tissue in the lesions would have been destroyed or at least much distorted, owing to the new-formed tissue pushing the elastic fibres aside. The significance of this particular type of reaction has been explained by Aschoff (1924) and Ranke (1928) as the typical response of the tissues in a non-infected, non-allergic organism. Ranke further stated that the exudative type of lesion of the primary infection was rapidly followed by productive lesions surrounding the primary focus which became rapidly encysted, while similar changes were also present in the related lymphatic glands. On the other hand, Krause (1927) found the initial response in the subcutaneous tissue of the non-allergic guinea-pig to be formative or productive, while that of the allergic animal was exudative. It must be remembered, however, that different tissues and different animals may react differently to a first infection. Allergy, while/

while an important factor in determining the type of tissue reaction, is, however, not an exclusive one, and there appear to be many modifying circumstances such as the amount of re-infecting dose, the virulence of the bacilli, the resistance of the patient, and the localisation of the lesion. It does not explain all the facts, and at the present time the relation between it and the type of tissue reaction is often obscure.

What was the relationship of the focus to the diseased tracheo-bronchial glands? Foci without related diseased glands were seldom found, Gohn describing only one case in a series of 170 with lung foci. The lymphatic glands draining the region in which the primary focus was situated were practically in all cases the seat of a tuberculous lesion of the same age or younger than that in the primary lesion in the lung. According to Engel (1923), the hilum nodes related to the primary focus became involved earlier, and were relatively larger in the infant than in older children. All these authors were agreed that by examining the glands at the hilum of the lung they could predict, with a reasonable degree of certainty, in what lobe the primary lesion was likely to be found. Often the path of infection could be traced from the primary lesion through the intra-parenchymatous group of the broncho-pulmonary glands to the superior or inferior/

inferior tracheo-bronchial glands at the hilum. The superior tracheo-bronchial along with the extra-parenchymatous broncho-pulmonary glands were generally first affected when the lesion was in the upper lobes. The inferior tracheo-bronchial were first involved when the focus was in the lower lobes or in the middle lobe on the right side. In the later stages, when there had been more extensive lymph node involvement, the tracheo-bronchial glands on the side of the lesion showed the more advanced change as compared with those on the other side, this being particularly so with the broncho-pulmonary glands which, as we have already pointed out, have no inter-communicating lymphatics with those of the other lung. In the tissue between the various groups of glands, intra- or extra-pulmonary, there was seldom any gross evidence of a tuberculous lymphangitis. Often the intra-parenchymatous glands showed no lesion, though the glands at the root were diseased. This was accounted for by the situation of the focus which, as we have already noted, was found for the most part just under the pleura, and so the lymphatic drainage from the focus might take place through the pleural plexus. This would be all the more likely if the deep lymphatics of the lung had become obstructed by the lesion, as then the small lymphatic channel already mentioned which formed an anastomosis between the superficial and/

and deep lymphatics of the lung would be utilised for drainage into the pleural plexus, through which the infection would be conveyed to the broncho-pulmonary glands at the hilum. Further, as is well-known in cases where there is a spreading tuberculous adenitis the infection may occasionally pass through one or more glands without producing any lesion recognisable by the naked-eye.

Most of these authors found that there was little, if any, retrograde spread from the diseased glands into the lung substance. A tracheo-bronchial gland might become adherent to a bronchus, however, and rupture into it, setting up a tuberculous broncho-pneumonia, but such an event was rare. For the most part the disease in the glands spread upward along the para-tracheal glands to the upper part of the thorax, the intensity of the tuberculous adenitis diminishing as the lesion passed upwards, i.e., as the distance from the primary focus became greater. This observation was of importance as it indicated the site of the original infection. Sometimes the disease had spread into the lower deep cervical glands, and occasionally there was a retrograde spread to the glands in the lower mediastinum, and from there through the diaphragm to the glands in the upper part of the abdomen around the coeliac axis and pancreas.

In the few cases where the tracheo-bronchial glands were/

were tuberculous and no primary lung focus was found, many of these authors accounted for such as having been due to the primary lesion in the lung being so small that it was missed, or that it had healed, or that the bacilli had passed through the bronchial mucosa, as in the case of the intestinal, without leaving any, or only a microscopic trace of its passage. In fact, Ranke mentions one case where the primary lesion consisted of a small, calcified patch in the bronchial mucous membrane. The above authors seldom failed to find the primary lung focus, however, Parrot always finding it in his series of 145 cases, while Gohn failed to find it only 7 times out of 177 cases in which the infection was primarily in the respiratory system.

Thus, according to these authors, it appeared reasonably certain from the various aspects of morbid anatomy, histological appearances, and the different responses of native tissue as compared with the allergic, that what they had described as the primary lung focus was indeed such, and that it was the result of inhalation of the tubercle virus. One thing alone remained to more or less complete the proof of this, namely, the bacteriology of such lesions. This we have attempted in the present investigation. It seemed to us that conditions in Scotland would provide a suitable field for such work as/

as the incidence of infection with the bovine virus in this country had been found to be high by various workers including Fraser (1912), Mitchell (1914), Griffith (1915 and 1930 (2)), Tulloch, Munro, Ross and Cumming (1924), Munro and Cumming (1926), Munro (1930). If the primary focus in the lung and associated lesions in the tracheo-bronchial glands were of haematogenous origin, then a high percentage of bovine infections would be found, but, as we shall prove, this was not so. It was necessary, however, to study the morbid anatomy and the histology of the tuberculous lesions met with in order to be sure that what we regarded as the primary complex was the same as had been described by other authors. The results of this study follow.

Methods of investigation in the author's cases.

At post-mortem, when the thoracic cavity was opened, a general survey of the organs, pleural cavities and lymphatic glands was made before the organs were disturbed. Any adhesions between the visceral pleura and the parietes were noted. The pericardial sac was then opened, and the heart removed so as to allow more room for further examination of the glands around the bifurcation of the trachea, and/

and also of the medial aspects of the lungs. The cervical region was next searched for any evidence of glandular disease, after which the structures of the neck were dissected out and removed along with the whole of the thoracic contents which were also carefully removed as far down as the level of the diaphragm. When any strong adhesions that could not be easily separated were present between the thoracic viscera and the parietal tissues, the latter, e.g., pleura or diaphragm, were cut into and removed along with the specimen so that the lungs were not damaged by any forcible separation. This, however, was not always possible where the adhesions were very dense and widespread. Careful dissection was however desirable in view of the fact that the primary lesion might be in the sub-pleural region of the lung in relation to these adhesions which, if forcibly torn through, might also tear out the lesion. The whole specimen was now subjected to further detailed investigation. All the superficial aspects of the lungs were carefully inspected, including the interlobar sulci, and often the primary focus was noted during this examination. Such superficial foci frequently projected very slightly from the surface of the lung. When any localised adhesion had been noted, either when the organs were in situ or during removal, the area where the adhesions were situated was subjected to special investigation. This often revealed/

revealed a caseous sub-pleural primary focus. If superficial inspection failed to reveal the primary lesion, the lungs were carefully palpated with the fingers, and any resistant nodular area cut into. In a few cases these resistant areas were found to be due to the primary focus.

The glands in the region of the bifurcation of the trachea were now examined. It was found easier to do this from the posterior aspect after removal of the oesophagus and aorta than from the anterior. Any evidence of enlargement or disease in the substance of the glands was noted, and the exact anatomical position of the affected glands determined. In some cases this was easy, as the tuberculous glands on one side only were involved, and often the superior showed a more advanced lesion than the inferior, or vice versa. From the bifurcation of the trachea the glandular lesion was traced back into the extra-parenchymatous broncho-pulmonary lymph nodes, and the extent of the lesion in these on one side compared with any in the corresponding glands on the opposite side. If the primary focus had not previously been discovered, these observations generally narrowed down the search to one lung, and, indeed, often to one lobe. At other times the tuberculous disease in the tracheo-bronchial glands was more extensive, and in these cases an attempt was made/

made to distinguish on what side and in what particular group of glands the lesion was most advanced. In these difficult cases the condition of the broncho-pulmonary glands was of much help in deciding the side which had been primarily involved. These glands, as we have already noted, have no communication with those on the other side, and where they were the seat of extensive tuberculous disease on one side only we invariably found that it was on this side that the primary focus was present. A cut was now made into the lung substance radiating out from the hilum to the middle of the focus in those cases where the primary lesion had already been discovered either by superficial examination or by deductions made from the group of glands which was the most involved. The condition of the lung between the focus and the hilum was now noted, particular attention being paid to the condition of the intra-parenchymatous lymphatic glands. In other cases where the focus had not been localised, and where the condition of the glands at the roots pointed to a particular area either in a lobe or a lung as the likely site of such a lesion, sections were made extending out from the hilum through the middle of each lobe to the lateral border. The cut surface was carefully inspected and palpated, and any lesion in the lung substance noted. Such a procedure often located the/

the lesion, but at other times many other such sections had to be made parallel to the ones already existing. Each slice of lung tissue was carefully examined and palpated, and occasionally as long as half-an-hour was spent in an endeavour to locate the primary lesion. The greatest difficulty was experienced in the neighbourhood of the hilum of the lung where the presence of the larger bronchi made palpation less satisfactory. The larger bronchi and trachea were now opened to see if there was any sign of ulceration of diseased glands into the bronchi. Lastly, the extent of the tuberculous adenitis in the para-tracheal glands on either side of the trachea was noted. Any spread of the tuberculous disease upward into cervical glands or downward into the lymph nodes in the lower mediastinum, and from there into the abdominal lymph glands, was also looked for.

In the recording of these cases it was found helpful to use blank figures of the lungs, and to fill in the lesions observed in these organs as well as the extent of the glandular involvement. Many of these figures are shown later. In fig. 3 the various symbols used in these diagrams are given, but in addition to this we noted at the side of the charts the nature of the lesions present in the lungs.

Results.

Of the 1,300 autopsies studied 216 showed tuberculous lesions, and of these we considered that the pathological evidence was in favour of the oldest lesion being in the thorax in 123 (56.9 per cent.). These thoracic cases were divided into two categories:-

A. Those in which a primary focus was found in the lung. In this group there were 102 cases.

B. The remaining 21 cases where no such focus was found. These were considered doubtful thoracic cases.

A. Cases with primary lung foci.

Number of foci.

Before describing the general characters of the lesion which we considered primary, the number of foci present in each individual case will be described. On summarising the 102 cases in which foci were present the following distribution was found:-

84 cases had 1 lung focus each.
11 " " 2 " foci "
5 " " 3 " " "
2 " " 4 " " "

From/

From this it is seen that in 18 cases multiple foci were present in the lungs. In a few of these cases all the foci were not primary, as some were undoubtedly younger than others. Thus the number of cases with multiple primary foci can be reduced, and further description simplified. In this elimination of secondary foci the following factors were considered:-

(1) The morbid appearances of the foci. Was there any evidence of surrounding fibrosis or calcification? If such existed in one focus and not in another, an important point regarding the age of the lesion was obtained.

(2) The changes in the related lymph glands. This chiefly applied to foci in different lungs, though occasionally also in different lobes. When the glands related to one focus showed a more advanced stage of tuberculous disease than those related to another, then the focus related to the glands showing the least morbid change was probably younger than that related to the nodes showing more advanced disease.

(3) The changes in the pleura. This, of course, only applied to foci which were situated immediately under the pleura. If there was a chronic pleurisy over one focus and not over another, then the former was regarded as probably the older.

(4) <u>The size of the focus</u>. This was considered as relatively unimportant. In cases of rapidly advancing tuberculous broncho-pneumonia where fusion of neighbouring lesions had taken place, size was likely to lead to a false conclusion.

(5) <u>The histological appearances of each of the foci</u>. Was there any evidence of early fibrosis or secondary tubercles at the edge of the focus, or early deposit of lime salts in the centre?

It was obviously not possible to make all these essentials apply to each focus. Before a decision was made, however, as to which focus or foci were primary in cases where there were multiple foci as many of the above factors as were possible were considered, and a differential diagnosis was never made unless histological evidence was also available.

Returning, now, to consider the 18 cases with multiple foci, it was found that in 14 of them the tuberculous disease was of such a nature as to cause multiple large lesions in the lungs. In 4 of them the lesions were primarily characteristic of chronic peri-bronchial tuberculosis where, on account of the chronicity of the lesions, interpretation was difficult. In passing, we may remark that 3 of these children were over 8 years of age, while one was/

was aged 4 years and 9 months. In none of these cases was the chronic tuberculous disease limited to one lobe, and often more acute secondary lesions were present. In another 10 cases the lesions were of a tuberculous broncho-pneumonic nature. In the remaining 4 children, isolated multiple chronic foci were found either in the same lobe or in different lobes of the lungs. In figures 4 to 6 some typical examples are given, and the various factors noted which aided us in arriving at a conclusion as to what should be regarded as the primary site or sites of infection. By taking into account the essentials which we have described above for the differentiation of a primary focus from a secondary one, due either to an endogenous or exogenous infection, the number of cases with multiple foci was reduced to 11. Accordingly, we considered the actual distribution of primary foci as follows:-

91	cases	had	1	primary	focus.
8	"	"	2	"	foci.
2	"	"	3	"	"
1	"	"	4	"	"

Thus in 102 cases there were 117 primary foci, and the general characters of these will now be considered.

Appearance/

Appearance of the primary lung foci.

p.123

In table XII the size and pathological characters of the various primary foci as noted at autopsy are summarised. While the post-mortem appearances gave a general idea as to the character of the lesions, yet, as we shall see later, when the histological details were considered in a small series of foci it was found that often the earlier stages of the healing process, of early breaking down of caseous lesions, and of early deposit of calcium salts were not observed on naked-eye examination. Indeed, these changes were so fine that it was impossible to make such observations from the morbid anatomical appearances. We considered it desirable, however, to discuss the macroscopic findings separately, as by doing so we would be in a better position to compare our findings with those of other workers, as it was evident at an early stage in this investigation that the lesions with which we were dealing were different in many respects from those found in children in other countries.

(1) Size.

In judging the size of the various foci, the secondary tubercles which often surrounded the primary focus were not taken into account. These were present, particularly at the edge of foci showing caseation or cavitation/

TABLE XII. The size and macroscopic characters of the primary lung foci.

Character.	Size.						Total.
	Less than pea-sized	Size of pea.	Size of bean	Size of hazel-nut.	Size of walnut	Size of plum and larger.	
Caseous	2	9	3	7	2	1	24
Caseous with breaking down.	-	5	4	8	4	1	22
Caseous with fibrosis around.	3	10	3	6	1	-	23
Caseous with calcification with fibrosis around.	-	3	-	2	1	-	6
Cavity with caseous walls.	-	7	5	8	7	7	34
Cavity with caseous walls with fibrosis around.	-	1	1	4	2	-	8
Total	5	35	16	35	17	9	117

cavitation, and were undoubtedly due, as histological observations later proved, to a lymphogenous spread from the primary lesion. As shown in table XII, which lays no claim to mathematical accuracy, the great majority of the foci were small. Gohn also noted this. Slightly more than three-quarters of the foci (91 out of 117) which we found were about the size of a hazelnut or smaller, while larger foci, walnut- and plum-size, composed less than a quarter of the whole group. These larger foci were found mostly in young children in whom the lesions were of the nature of a tuberculous bronchopneumonia, and, to a less extent, in older children suffering from chronic forms of phthisis, with cavitation.

(2) Caseation.

It was not an easy matter to separate purely caseous lesions from those showing evidence of breaking down, and accordingly the figures given in table XII are probably not strictly accurate in this respect. When the caseous matter in the lesions was softer than usual in consistence, showed a ragged, fissured surface on section, and was easily washed away by a gentle stream of water, then such evidence was considered sufficient for classifying the caseous lesions as showing early breaking down. It is of interest to note that our opinion in this matter was often supported/

supported by other evidence. For example, in some of these cases tubercle bacilli were actually found in the sputum obtained in children by the method described by Findlay (1926). Also, in many of these cases with signs of breaking down of the caseous focus, small acute tuberculous ulcers were found in the upper part of the intestine due to infection by sputum that had been swallowed. This relationship of the lung disease to lesions in the bowel will be more fully considered later. Of the 117 foci 24 were purely caseous, 22 showed early softening of the caseous material, while all the remainder were the seat of caseation in addition to some further pathological change. Under these later alterations the remaining primary lesions have been classified.

(3) Fibrosis.

At the autopsies fibrosis was evident when the lesions were more sharply marked off from the surrounding lung tissues. Such lesions often showed a surrounding zone of reddish-brown induration, and occasionally when near the surface of the lung actual puckering of the pleura was observed. Nearly all these observations, however, were confirmed microscopically, and, as we shall discuss later, patchy fibrosis was found on microscopic examination at the edge of some of the very early lesions. It was not possible/

possible, however, to diagnose these early stages of fibrosis from the post-mortem appearances until the process was fairly well advanced. As will be seen from table XII, fibrosis was observed around 23 caseous, 6 partly calcified foci and 8 caseous-walled cavities, that is in slightly less than one-third of the lesions (37 out of 117, or 31.6 per cent.). No completely fibrosed focus was observed in our series. Such "scar" foci have, however, been noted by some of the continental workers mentioned above.

(4) Cavitation.

As evidence of cavitation a definite breach in the continuity of the lung parenchyma was considered essential. The cavities were mostly of a larger size than the solid lesions, as shown in table XII. No completely shrunken cavities were noted in the whole investigation.

(5) Calcification.

It was found very difficult to decide at what stage a lesion could be considered as being the seat of calcification. When the lesions no longer cut cleanly with the knife, or offered some resistance, and when the central area had a gritty feel or a chalky consistence such were considered as showing this change. In all of these lesions this/

this naked-eye evidence was confirmed by histological examination, and as we shall discuss later the deposit of calcium salts in small amounts, as shown by microscopic examination, occurred in many lesions before it was possible to appreciate this by naked-eye examination. No completely calcified lesion, however, was present in any of the specimens which we have examined. Lesions showing evidence of macroscopic calcification were rare in our series, constituting just over 5 per cent. (6 out of 117) of all the primary lesions. This is in marked contrast to the observations of continental and American writers, some of whom, on account of the calcification of the foci, have been able to demonstrate these radiologically during life (Opie (1917), Stoloff (1927)). This, however, was not the case with any of the foci which we found.

The age distribution of the foci.

In table XIII the naked-eye characters of the primary foci found are summarised according to age groups. Those with no evidence of repair are tabulated first, and secondly those in which some evidence of healing was present (fibrosis and calcification). During the first 3 months of life relatively few primary lung foci were met with, only 4 being observed in the present series.

These/

These foci were found in 3 children, one of whom had 2 primary lesions. The youngest case showing these foci was a boy, aged 7 weeks, in whom two foci were found in the lungs in addition to a widespread sub-acute generalised miliary tuberculosis and extensive tuberculous adenitis of the lymph nodes in the thorax and upper part of the abdomen. There was no family history of tuberculosis, and no evidence of pulmonary tuberculosis was found either radiologically or clinically in the father or mother. The child, however, lived in a one-roomed house, with other people, and it was possible that the source of infection may have been here, though we were unable to find out if this actually was the case. As this case is of interest, it is described in fig. 7. The earliest age at which definite cavities were noted was in a male child aged 17½ weeks whose mother had died 5 weeks before of pulmonary tuberculosis. This case is described in fig. 8.

It will be observed from the table that only one focus (2 per cent.) out of the 50 in the first year of life showed any naked-eye evidence of healing. This was found in a female child, aged 7½ months (32 weeks), in whom a caseous area the size of a nut was found in the upper lobe (fig. 9). Around this area there was some induration, and the pleura showed slight puckering. Unfortunately/

TABLE XIII. Age distribution of primary foci according to their macroscopic characters.

Character.	0 to 3 months	3+ to 6 months	6+ to 9 months	9+ to 12 months	12+ to 18 months	18+ to 24 months	2+ to 3 years	3+ to 4 years	4+ to 5 years	5+ to 6 years	6+ to 7 years	7+ to 8 years	8+ to 9 years	9+ to 10 years	10+ to 11 years	11+ to 12 years	Total.
Caseation.	4	8	6	5	8	6	8	1	-	-	-	-	-	-	-	-	46
Cavity with caseous walls.	-	8	8	10	4	2	1	-	-	-	1	-	-	-	-	-	34
Total lesions show no healing.	49				20		9	1			1						80
Caseation with fibrosis.	-	-	1	-	1	2	5	-	1	3	3	1	3	1	1	1	23
Caseation with calcification with fibrosis.	-	-	-	-	-	-	-	-	3	-	-	-	2	-	1	-	6
Cavity with caseous walls with fibrosis around.	-	-	-	-	-	4	1	-	1	-	1	-	-	-	1	-	8
Total lesions showing evidence of healing.	1				7		6		5	3	4	1	5	1	3	1	37

Unfortunately, the importance of this finding was not recognised at the time, and the focus was used for isolation of the infecting virus so that the naked-eye evidence was not confirmed microscopically. During the second year of life the proportion of foci which showed evidence of healing was greater; 7 out of 27, or 25.9 per cent., showed some naked-eye evidence of fibrosis. It was at this period, too, that the first evidence of an attempt at encapsulation of cavities was noted. This case is of interest as it occurred in a male child, aged 1 year and 11 months, in whom four small cavities were present in the left upper lobe (fig. 10). To the naked-eye these were surrounded by an indurated area, and this was confirmed microscopically as being due to fibrosis. In addition there was marked chronic pleurisy on both sides, and chronic adhesive pericarditis, with consequent atrophy of the heart. After the second year the proportion of cases which showed macroscopic evidence of healing rose rapidly, there being 6 out of 15 (40 per cent.) in the third year of life in which this change was present. After that period the numbers became too small for any comparison to be made of the various changes in groups of one year. As will be seen from table XIII, however, 23 out of the 25 lesions found after 3 years of age showed naked-eye evidence/

evidence of an attempt at arrest of the disease. It was only between the fourth and fifth years that calcification could definitely be said to be present in the lesions seen at autopsy. The earliest age at which this was noted was in a child aged 4 years and 9 months, in whom a chalky, pea-sized focus was found near the hilum in the upper lobe. Calcification has been noted at a much earlier age by continental workers; Geipel (1906) observed it in two children aged 6 and 8 months, Schürmann in a child aged 9½ months, Kuss at 11 months, and Gohn at 17 months. There was no case in this series in which the anatomical evidence was in favour of complete healing and arrest of the tuberculous disease in the focus.

<u>The situation of the primary foci</u>.

The situation of various primary foci in the different lobes of the lungs is summarised in table XIV. The right lung was found to be the seat of the primary focus more often than the left. Of the 117 foci considered primary, 80 (68.4 per cent.) were on the former side, while 37 (31.6 per cent.) were on the latter. The upper lobes on both sides were more frequently involved than the lower, while the right upper and right lower respectively were more often the seat of the primary/

TABLE XIV. The distribution of the primary lung foci in the various lobes.

No. of foci.	Situation of the foci. Lung.	Lobe.			Percentage of total No. of primary foci.
117	Right 80 = 68.4 per cent.	Upper lobe	(Superficial, 27) (Deep, 19)	46 = 57.5 per cent.	39.3
		Middle lobe	(Superficial, 8) (Deep 3)	11 = 13.75 per cent.	9.4
		Lower lobe	(Superficial, 13) (Deep, 10)	23 = 28.75 per cent.	19.6
	Left 37 = 31.6 per cent.	Upper lobe	(Superficial, 16) (Deep, 5)	21 = 56.8 per cent.	17.9
		Lower lobe	(Superficial, 9) (Deep, 7)	16 = 43.2 per cent.	13.7

primary lesions than the corresponding lobes on the left side. As regards the frequency of the primary lesion in the various lobes, we found the right upper lobe the seat of the greatest number, and then in order the right lower, left upper, left lower, and right middle. Thus the right upper lobe was found to contain the greatest number of foci, and the middle lobe the least. When the cases with multiple primary foci were considered alone, we found that in 8 cases where there were 2 primary foci, both lungs were involved in 4. In 5 of these cases the lesions were in different lobes. In the 3 cases with more than 2 foci these were limited to the one lobe. In the following table our results are compared with those of Gohn.

Author.	No. of foci.	Right lung.			Left lung.	
		Upper lobe.	Middle lobe.	Lower lobe.	Upper lobe.	Lower lobe.
Gohn	200	57	14	39	50	40
	Percentage to total No.of foci	28.5	7.0	19.5	25.0	20.0
Blacklock	117	46	11	23	21	16
	Percentage to total No.of foci	39.3	9.4	19.6	17.9	13.7

Thus we are in agreement regarding the greatest incidence of the primary lesion in the right upper lobe. In Gohn's cases, however, the left upper lobe came next in order of frequency, while in ours it was the right lower. In both series the right middle lobe was least involved. Kuss, on the other hand, found the lesions more often in the lower lobes, both of which were equally affected. Schürmann, working chiefly with subjects over 18 years of age, found the relative frequency of involvement of the lobes similar to Gohn.

An attempt was made to find the commonest site of the lesion in the various parts of the different lobes. For this purpose each lobe was divided into anterior, posterior and medial aspects, these being further subdivided into upper, middle and lower thirds. In table XV and fig. 11 the number of foci found on these various surfaces has been summarised. The numbers in the table are, however, not strictly accurate, as of the 117 primary foci found 73 were situated immediately under the pleura, while the remaining 44 were situated a short distance below, but generally nearer one surface than another. The position of these latter lesions has been charted according to the surface to which it was considered they were nearest. This greater frequency of the superficial position of the foci has also been noted by/

TABLE XV.

Distribution of the primary foci in the various parts of the lobes. (Compare fig. 11).

Lobe.	Aspect	Upper third	Middle third.	Lower third.	Total.
Right upper	Anterior Medial Posterior	17 1 1	10 2 2	10 - 3	37 3 6
	Total	19	14	13	46
Left upper	Anterior Medial Posterior	3 - -	9 2 1	5 - 1	17 2 2
	Total	3	12	6	21
Right lower	Anterior Medial Posterior	3 - 1	1 1 4	3 2 8	7 3 13
	Total	4	6	13	23
Left lower	Anterior Medial Posterior	2 - 3	1 2 1	1 - 6	4 2 10
	Total	5	4	7	16
Right middle	Anterior Medial Posterior	3 - -	5 1 2	- - -	8 1 2
	Total	3	8	-	11

by Gohn, by Kuss, and by Gohn and Winternitz (1924). It will be appreciated from table XV that the surfaces most frequently involved were the anterior in the case of both upper lobes and of the middle lobe, while in the lower lobes it was the posterior. As to the commonest position of the lesions relative to these surfaces, the upper third of the right upper lobe was most frequently involved, the middle third of the left upper and right middle lobes, and the lower thirds (i.e., the bases) in the case of both lower lobes. It is to be noted, however, that in the case of both upper lobes the actual apex of the lung was seldom affected, only 2 cases being noted in the present series. In these results we are in general agreement with Gohn, apart from the fact that this author found that the upper and lower thirds of the two upper lobes were almost equally affected.

To sum up, then, we found the primary foci most often in the various lobes in the following situations:- the upper third of the anterior aspect in the case of the right upper lobe; the middle third of the anterior aspect of the left upper and right middle lobes; and the lower third of the posterior aspect of both lower lobes.

The distribution of the tuberculous disease in the tracheo-bronchial glands in cases with primary lung foci.

In the first place, only the 84 cases with one lung focus/

focus each will be considered. The remaining 18 cases with multiple primary and secondary foci will be discussed later.

Right upper lobe. In this lobe we found 38 single primary foci, and the spread of the tuberculous lesions to the neighbouring tracheo-bronchial glands is shown diagrammatically in fig. 12 where the numbers corresponding to the position of the different groups of glands indicate the number of cases in which these glands were affected. It is to be noted that in all the cases the right superior tracheo-bronchial group was always involved as well as the extra-pulmonary nodes of the broncho-pulmonary group, while the intra-pulmonary nodes in this group were only involved in 14 cases. Only in 3 cases was the lesion confined to the right broncho-pulmonary glands and the right superior tracheo-bronchial. In fig. 13 a case with a single primary focus in the right upper lobe, with a typical distribution of the glandular lesions, is shown.

When several groups of glands were involved, the tuberculous adenitis diminished in severity the more distant it became from the primary lesion, the lesions in the right superior group of glands being always more advanced and more extensive than in any of the other groups. For/

For example, in cases where the primary focus was in the right upper lobe, and where the left inferior tracheo-bronchial glands were involved, the lesion in the latter was less marked and less extensive than that in the right superior group. Further, the right broncho-pulmonary glands related to the lower and middle lobes were sometimes the seat of a less marked lesion than those related to the upper lobe. The intra-parenchymatous glands in the lower and middle lobes were never affected when the primary lesion was in the upper lobe, and the broncho-pulmonary nodes on the left side were always free of any lesion in these 38 cases with the primary focus in the right upper lobe.

It is of interest to note that in the 16 cases where the right inferior group of glands was tuberculous, the primary lesion was found in the lower third of the upper lobe in 9, in the middle third in 5, and in the upper third in 2. These results are not surprising in view of the findings of Most which have already been discussed in the section dealing with the anatomy of the pulmonary lymphatic system. In the case of these foci in the lower parts of the upper lobe, the infection may have passed through the pleural lymphatics, then into the lymphatic channels in the interlobar fissure, and so to the lower tracheo-bronchial glands. This would be more likely/

likely to occur if the deep lymphatic system to the hilum was obstructed by the tuberculous lesion,as the communicating channel between the deep and pleural lymphatics would then be used. Of course the infection may have passed directly from the superior to the inferior group of glands, but the fact that the latter group was the seat of a tuberculous adenitis in 9 out of 11 cases with single foci in the lower third of the upper lobe and in only 2 out of 16 similar cases in the upper third is suggestive that the infection probably spread to the inferior group of glands through the lymphatics in the interlobar sulcus as described. In 3 of the cases with the primary lesion in the right upper lobe, the tuberculous adenitis had spread down through the right inferior tracheo-bronchial glands to those in the lower mediastinum, and from there through the diaphragm to the lymph nodes in the upper part of the abdomen. This was a retrograde spread, and such must be expected where there is any extensive blocking of lymphatics by tuberculous or other disease. For example, in cases of chronic interstitial pneumonia with, in addition much anthracosis and chronic inflammatory fibrosis in the tracheo-bronchial glands, we have noted in the adult that the glands in the upper part of the abdomen sometimes contained carbon pigment. The/

The extent and severity of the tuberculous lesions in the left para-tracheal glands were always much less than in those on the right, and on passing upwards the diminution of the morbid process was always more rapid on the left side, never extending to the deep cervical glands in the lower triangle of the neck as was found on 12 occasions on the right. The lesions in the right deep cervical glands in the lower part of the neck were never extensive, and generally consisted of a few caseous points scattered throughout the substance of the glands. A case of this nature is described in fig. 14. Widespread tuberculosis of lymph glands was more often met with in cases where the primary lesion was large, or where there were severe secondary lesions, e.g., in cases with cavities complicated with secondary tuberculous broncho-pneumonia, and in fig. 15 an example of such a case is given.

Left upper lobe. In the case of the left upper lobe, 12 solitary primary foci were found, and the distribution of the lesions in the lymphatic glands in these cases is summarised diagrammatically in fig. 16. A primary focus in the left upper lobe caused tuberculous adenitis in the same groups of glands on the left side as a primary lesion in the right upper lobe did on the right, but there was relatively less extension to the lower deep cervical glands on/

on the left side as compared with the right, and in addition no extension to the abdominal lymph nodes was observed. The intra-parenchymatous glands were involved in 5 of the cases, while the broncho-pulmonary at the hilum were involved in all, the lesion generally being most marked in those glands along the bronchus to the upper lobe. The left superior tracheo-bronchial glands were the seat of tuberculous disease in all the cases. In only 2 cases was the tuberculous adenitis confined to the left broncho-pulmonary and superior tracheo-bronchial. As on the right side, it was found that cases with severe or extensive pulmonary lesions caused tuberculous adenitis in all the groups of tracheo-bronchial glands, with the exception of the right broncho-pulmonary group. In fig. 17 a case of a focus in the left upper lobe, with a typical distribution of the glandular lesions, is shown, while for comparison in fig. 18 a case with more wide-spread glandular disease is given.

Right lower lobe. In the right lower lobe there were 14 cases with one focus each. The extension of the lesion to the tracheo-bronchial glands, and the degree of involvement of these structures are shown diagrammatically in fig. 19, from which it is seen that in all but one instance the right inferior group of tracheo-bronchial glands was/

was the seat of tuberculosis. In the one exceptional case, it was the right superior tracheo-bronchial group and not the inferior in which the tuberculous adenitis was found, as is described in fig. 20. In this case the primary lesion was found in the sub-pleural region on the posterior surface near the apex of the lower lobe. It would seem that the infection in this particular instance had travelled by way of the pleural lymphatics to those in the interlobar sulci, and through these to the upper tracheo-bronchial group. In all the cases the right broncho-pulmonary glands were involved. There was no spread of the glandular lesions downwards towards the abdomen in any of the cases, though in 3 there was a slight upward spread into the inferior deep cervical glands on the right side. These cervical nodes, however, were the seat of only a few caseous foci. As would be expected from the very free intercommunication by lymphatics existing between the right and left inferior tracheo-bronchial glands, the tuberculous disease had spread across from the glands on one side to those on the other in almost half of the subjects There was also a certain amount of spread upward to the right upper tracheo-bronchial glands on the same side, but only in one case were the superior glands on the opposite side affected. In fig. 21 a typical example is given of the spread of the tuberculous lesion from/

from a focus in the right lower lobe, while in fig. 22 a case with more extensive involvement of the glands is shown.

Left lower lobe. In the left lower lobe 13 single primary lesions were found, and the glandular lesions in these cases are shown in fig. 23. The left inferior tracheo-bronchial glands related to 11 of the foci were the seat of tuberculous disease, and in fig. 24 a typical case is shown. In 2 cases, however, the left broncho-pulmonary and left superior tracheo-bronchial glands were involved, while no naked-eye lesion was noted in the inferior group. The distribution of the glandular disease in these 2 cases corresponded to the one described above in connection with a focus in the right lower lobe. Both of these lesions with the unusual glandular involvement were situated posteriorly at the edge of the interlobar fissure, one in the upper third and the other in the middle third, so that there had been an opportunity for the infection passing through the lymphatics of the pleura to those in the interlobar sulcus, and so to the upper tracheo-bronchial glands. One of these cases is described in fig. 25. As in the case of lesions in the right lower lobe, the glandular disease had extended across to the inferior tracheo-bronchial on the other side in 5 out of the 11 cases, and also in a few cases to the superior tracheo/

tracheo-bronchial on the same side. The left para-tracheal glands were involved in a few instances, and from these there had been an extension of the disease into the lower deep cervical glands in 3 cases. Spread of the tuberculous disease had taken place in 2 of the children to the glands in the lower mediastinum, and from there through the diaphragm to the lymphatic glands in the upper part of the abdomen.

Right middle lobe. In the right middle lobe there were 7 cases with one primary focus each, and the relation of these to the tracheo-bronchial glands is shown in fig. 26. In six instances the right broncho-pulmonary and right inferior tracheo-bronchial glands were most involved, while in the remaining case the most extensive lesion was found in the right superior tracheo-bronchial glands, the inferior group showing no lesion to the naked-eye. In this case the primary lesion was in the upper part of the anterior aspect of the lobe at the edge of the interlobar sulcus. Accordingly, the distribution of the glandular lesion in this case corresponded with that found in 3 foci in the lower lobes which have already been described. This case with the atypical distribution of the glandular lesions is shown in fig. 27, while a typical case is given for comparison in fig. 28. As in the/

the case of the other lobes, in addition to the lesions in the right broncho-pulmonary and right inferior tracheo-bronchial, there was also some involvement of the neighbouring glands in nearly all the cases. Direct extension through the right para-tracheal to the deep cervical glands on the right side was noted in 2 cases.

Cases with multiple foci. Where the lesions were multiple in the same lobe, then the distribution of the glandular lesions corresponded to that found when there was only a single lesion. In other cases where the foci were in different lobes, then the extent of the lesion in the related glands corresponded to the age of the various foci, those glands related to foci of longer standing, and hence primary, showing more extensive tuberculous disease than those related to more recent lesions. While some estimation could be made regarding the ages of different foci from the related glandular lesions, yet in a few cases with multiple chronic or acute lesions no such distinction could be definitely made out. These cases we have already discussed in the section dealing with the number of foci.

The relation of the pathological changes in the primary lung focus to those in the associated tracheo-bronchial glands.

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In table XVI the pathological changes in the primary foci/

TABLE XVI.

The relation of the pathological changes in the primary lung foci to those in the associated tracheo-bronchial glands.

Pathological lesion in primary foci.	Pathological lesion in associated glands.
Caseation) = 46 Caseation + softening)	(Caseation........... = 34 (" + softening.. = 12
Caseation + fibrosis around = 20(a)	(Caseation........... = 10 (" + softening.. = 4 (+ fibrous peri- (adenitis.. = 6
Caseation + calcification + fibrosis = 6	(Caseation........... = 1 (" + fibrous peri- (adenitis.. = 2 (" + " " " (+ calcification = 3
Cavities with caseous walls = 30(b)	(Caseation........... = 15 (" + breaking-down = 15
Cavities with caseous walls + fibrosis around. = 5(c)	(Caseation........... = 2 (" + breaking-down = 1 (" + chronic peri- (adenitis.. = 2

(a) (b) (c). These figures are less than those given in table XII because in some cases with multiple foci these were all in the same lobe and hence acted like a single lesion in the production of pathological changes in the related lymphatic glands.

foci are summarised and compared with those in the associated tracheo-bronchial glands. It will be seen from this tabular summary that the pathological changes found in the glands were always at the same or at an earlier stage as compared with those in the foci. The glands never showed a more advanced morbid change. This was important because it proved that the focus was either of the same age or older than the lesions in the lymph nodes. This finding, as we shall see later, was confirmed when the histological lesions in the focus and related glands were compared.

To sum up, then, we found that the glands in direct anatomical relation with the focus were nearly always the seat of tuberculous adenitis. The few exceptions to this have been noted above and accounted for on anatomical grounds. When the tuberculous disease involved several groups of the tracheo-bronchial glands, those in direct anatomical relation to the focus showed the most advanced change. There was never any retrograde spread of the tuberculous disease into the lungs from the glandular lesions. For example, with a focus in the right upper lobe with extensive involvement of the tracheo-bronchial lymph glands, such as described in fig. 15, we never found that the intra-parenchymatous glands in the lower lobe on the same side were affected. When the broncho-pulmonary/

broncho-pulmonary glands on both sides were equally tuberculous then primary foci were found in either lung. As 80 (68.4 per cent.) of the primary lung foci were found in the right lung, the glands on that side were more frequently involved than those on the left. The most extensive glandular tuberculosis was found in relation to foci in the right upper lobe. Furthermore, the glands related to the primary lesion showed the same or an earlier morbid change as compared with that found in the primary lesion itself. The severity of the glandular disease always diminished the more distant the glands became from the primary focus. Thus it seems certain both from the anatomical and pathological findings that the tuberculous disease in the tracheo-bronchial glands was secondary to and caused by the primary lung focus.

<u>Histological observation on the primary complex</u>.

It will be appreciated that a complete histological investigation of the primary focus and related glands was not possible in the present investigation as tissue from one or both of those lesions was required for isolation of the infecting virus. Histological studies of what were considered primary foci were made in 67 cases; of these 41 were aged 2 years or less, while 26/

26 were over 2 years of age. These included all those in which there were multiple foci, all of which were examined microscopically to find, if possible, their relative ages. The lesions studied histologically consisted of those which for the most part showed no evidence of cavitation to the naked-eye. In only 15 of these cases were portions of the related superior or inferior tracheo-bronchial lymph nodes available for examination, as in the others this material had been used in the bacteriological portion of the work.

In the early stages of this investigation, we were impressed by the fact that some of the primary lesions were roughly wedge-shaped, the base of the wedge being towards the pleural surface. This shape was not unlike that met with in haemorrhagic infarctions in the lungs associated with old-standing mitral disease in the adult, and suggested the possibility that the primary lesion might have a vascular origin. Accordingly, in all the 67 cases the entire lesion was cut in serial sections,10 to 15 μ thick, and every tenth section examined. Having mentioned the possibility of a vascular origin, we may at once dismiss it by stating that we never once found evidence that the primary lesions were related to the vascular system. If such were found then the lesion was a secondary one. For example, in figs. 29a and 29b such a/

a case is shown. In this case, already described in fig. 5, 4 lesions were present, and on naked-eye examination there was some doubt as to which might be the primary one. On section, however, one showed some fibrosis at the edge, while the other lesions did not. In one of these latter, as shown in the figures, the vessel leading into the lesion was closed by endarteritis obliterans. Further along in the series of sections the cause of this endarteritis was found in the form of a miliary tubercle involving the outer and middle coats of the artery. On the other hand, we frequently saw the actual bronchiole leading into the focus plugged by caseous debris, and such a case is shown in fig. 30.

The sections in the serial were stained by haematoxylin and eosin, and where any special feature required further investigation, the sections on either side of the one showing the point of interest were stained by Gallego's modification of Mallory's stain or van Gieson, by Weigert's elastic tissue stain, by Ziehl-Neelsen, and by Gram's method. In all cases some sections in the series were stained by von Kossa's silver method for the demonstration of calcium salts. The delicacy of this micro-chemical test for the presence of calcium salts was considered important as it showed deposits of these at an early stage. If such could be found both in the primary/

primary lesion and related lymph nodes after staining by this method, and not by others, then an important point regarding the age relationships of the two lesions would be established. For example, in figs. 47a and 47b the former had been stained by haematoxylin, and from the appearance of the section it was very doubtful if lime salts were present, whereas in that stained by the silver method the presence of these was definitely shown. Only a few sections were made of the lesions in the lymphatic glands, and these were stained by the methods described above as required.

The youngest age at which a primary lesion was found was in a child aged 7 weeks (fig. 7). In this case the 2 primary lesions were of tuberculous broncho-pneumonic type. Extensive caseous change had taken place in both of these, and there was only slight cellular reaction around (small round cells, large mononuclears, a few endothelioid, but ~~no~~ giant cells), and no evidence of fibrosis. As the age of the children advanced, the lesions tended to become surrounded by fibrous tissue, and while this was the rule, yet there were some exceptions. The earliest age at which an attempt at definite fibrous tissue formation was observed on microscopic examination at the edge of the primary lesion was in the case described in fig. 21. This was a child aged

4/

4 months and the histological section from the edge of the lesion is shown in fig. 31. Yet in some older subjects, aged 8, 10, and 17 months, this was not noted. Evidence of the formation of fibrous tissue at the edge of the primary lesions was found in 8 (19.5 per cent.) out of the 41 children under 2 years, the lesions from whom were studied histologically. In all the 26 children over 2 years fibrosis was present at the edge of the foci, and encapsulation was more complete than in the younger children. Similar findings were noted in the case of early deposition of lime salts. The earliest age at which this was observed was in a child aged 6½ months. In this case a section of one of the tracheo-bronchial glands stained by von Kossa's silver method showed early deposit of calcium salts (fig. 32). No histological observations were made of the primary lesion in this case as it had been used for isolation of the infecting virus, - a human strain. As in the case of the fibrous tissue reaction around the foci, so the occurrence of lime salts also tended to be more frequent in the older subjects, but here again there were exceptions, and probably other factors must be taken into account. In 4 (9.8 per cent.) out of the 41 children under 2 years, and in 10 (38.5 per cent.) of the 26 over 2 years, there was microscopic evidence of/

of early calcification, either in the primary lesion or in the related lymph glands.

It appeared to us that progressive stages of the healing process could be followed in a series of primary foci, and a brief description of this follows. The earliest stage (fig. 33a) at which we found the primary focus from the histological point of view was typical of a localised patch of caseous broncho-pneumonia situated immediately under the pleural surface of the right upper lobe in a female child aged 7 months. In this case the air vesicles were filled with a cellular exudate (round cells, large mononuclears, a few polymorphs, and some plasma cells). In addition, the walls of the affected vesicles were swollen and infiltrated with similar cells. Caseous change had taken place in the central part of the lesion, and there was early softening. At the edge of this lesion (fig. 33b) there was some formation of young tubercle follicles in which there were a few giant cells. Some of the cells at the edge were fibrous tissue cells connected with which there was early formation of collagen fibrils. In such early primary lesions the bacilli were abundant, particularly at the edge near the cellular zone (fig. 33c), while in the central area where caseous change had taken place they were not so numerous (fig. 33d). These lesions/

lesions, on account of the large numbers of bacilli, were found most useful for isolating the infecting virus, and accordingly were frequently used. This early lesion, as will be seen from the low power figure, was fairly sharply demarcated from the surrounding lung tissue, and the microscopic appearance was different from that seen in an ordinary secondary broncho-pneumonic tuberculous lesion. The cellular exudate, with formation of tubercle follicles and evidence of early fibrosis at the edge of the lesion, was never met with in a broncho-pneumonic tubercle in a child arising as a secondary lesion in a case with cavity, as shown for comparison in fig. 34. In such a lesion there was little or no evidence of cell reaction, either in the affected air vesicles themselves or in those round about. A lesion of this nature was composed almost entirely of serous exudate in which there were few cells. Caseation was rapid, and the edge was ill-defined. From the histological appearances, it appeared to be spreading quickly, and there was little attempt on the part of the surrounding tissues to arrest the pathological process.

At a later stage (fig. 35a) the focus was completely caseous, and there were a few secondary tubercles around. At the edge of the caseous material the amount of fibrous tissue was greater than in the earliest stage, and there was/

was infiltration of small round cells and large mononuclears with well-formed tubercle follicles (fig. 35b). In the next stage the surrounding fibrosis had become greater and the lesion appeared more sharply marked off from the neighbouring lung tissue (figs. 36a and 36b). Around the numerous secondary tubercle follicles at a later stage there was fibrous tissue formation, and this, with that already noted at the edge of the focus, made the amount of surrounding fibrosis greater (fig. 37a). The encapsulating fibrous tissue which showed signs of contraction had become denser (fig. 37b), and now almost completely surrounded the focus. Finally, the whole lesion was surrounded by a well-formed fibrous capsule, as shown in fig. 38, and the secondary tubercles which were found immediately around the lesions at an earlier stage had nearly all disappeared. In the latest stage in which we found the focus it was well encapsulated, sharply marked off from the surrounding lung by well-formed fibrous tissue, and in addition there was early calcification, as shown in figs. 39a, 39b, and 39c. The scars, petrified and osseous foci described by continental authors were never met with in the present study. Throughout all the above stages we noted that the elastic fibres in the walls of the air vesicles were always demonstrable when the sections were stained by Weigert's elastic/

elastic tissue stain. Even in the lesions fairly well advanced in the healing stage, as demonstrated by the encapsulation with fibrous tissue of early deposit of lime salts, some of the elastic fibres were still stainable, as shown in fig. 40 which was taken from the same serial section as those in figs. 47a and 47b.

In the lung tissue immediately surrounding these foci, interstitial change was often present. In the case of the lesions which showed early evidence of fibrosis at the edge, this consisted of an infiltration of small round and larger mononuclear cells, as shown in figs. 41a and 41b, which were taken from a lesion at the extreme base of the posterior aspect of the right lower lobe. At a later stage, when the fibrous encapsulation of the lesion became more marked, there was often a moderate degree of chronic interstitial fibrosis extending into the lung substance (fig. 42). The pleura in the neighbourhood of the primary foci was often noted to be the seat of lesions in this histological study. Sometimes localised patches of caseous pleurisy around which there was fibrosis were found in the visceral pleura, as shown in fig. 43, where the end of the lung focus is seen as a small darkly staining triangular area under the pleural lesion. In older foci the pleura immediately above showed much fibrous thickening (fig. 44). Both figs./

figs. 42 and 44 were from the same focus, the one showing the pleural surface and the other the deep surface in the substance of the lung. On both these aspects of the lesion there was well-marked fibrous tissue formation. Where pleural adhesions were present in the later stages in the neighbourhood of the focus, these were composed of fibrous tissue, and the spread of the tuberculous disease from the lung lesion could sometimes be traced into these adhesions, and so to the parietal pleura, as seen in fig. 45. The section shown in this figure was a little further along in the same serial section as that shown in fig. 37a. Evans (1908) has shown that definite lymphatic channels are formed in these adhesions, and through such channels this author has traced the spread of malignant cells to the thoracic parietes. The same would appear to hold equally good for the spread of tuberculous disease from the lung substance to the parietal pleura.

The histological observations also helped us to confirm whether the primary lesion and related lymph glands showed the same stage of the pathological process. This observation was only possible, however, in 15 cases. In all of them we found that the microscopic picture confirmed the findings we had already made by the naked-eye, viz., that the lesions in the primary focus and related lymph/

lymph glands were of the same age, or that the lesions in the glands were younger than those in the primary foci. If there was fibrosis around the lesion, then the glands generally showed evidence of fibrous peri-adenitis, as demonstrated in figs. 46a and 46b, where sections from an outgrowth of the primary lesion and related tracheo-bronchial glands in the case described in fig. 6 are shown. If the lesion showed early deposit of lime salts as demonstrable by von Kossa's silver method, then the related glands nearly always showed a similar lesion. Such a case is shown in figs. 47a, 47b, and 47c, where both the primary lesion and glands showed early calcification. In this particular case these deposits, as shown in fig. 47a, were scarcely recognisable when the sections were stained by methods other than von Kossa's silver method. This evidence of early calcium deposit in both the primary focus and related lymph glands was considered most important as it showed that the age of the lesions was the same. Further work on another series is being done on this subject of calcium deposit in tuberculous lesions in children. Where there was fibrous peri-adenitis around the enlarged caseous glands at the hilum, we found that the neighbouring air vesicles and small bronchi were collapsed. In addition there was some chronic interstitial change in the lung substance/

substance in the immediate neighbourhood, as shown in fig. 48. Such a lesion often affected quite a large area of tissue at the root of the lung.

Secondary lesions found in cases with primary lung foci.

(1) Lung substance. In the following table the lesions found in the lungs in the cases where there were primary foci are summarised:-

Lesions.	No. of cases.
Acute miliary tubercles	11
Sub-acute miliary tubercles	28
Acute broncho-pneumonic tuberculosis	11
Chronic peri-bronchial tubercle	4
Mixed miliary and broncho-pneumonic lesions	38
No other lesion except primary focus (pleural change in 7)	10
	102

It will be seen that in the majority of cases the associated lesions were of a mixed type (sub-acute miliary and early tuberculous broncho-pneumonia). To the naked-eye, the sub-acute miliary lesions were slightly larger than pinheads, round in shape, caseous, and stood out from/

from the cut surface, while the broncho-pneumonic lesions, on the other hand, were larger, more irregular in shape, and sometimes a bronchiole could be made out in the centre. There was often much difficulty, however, in distinguishing by the naked-eye between an early broncho-pneumonic lesion and a sub-acute miliary one, and for a definite diagnosis histological examination was frequently necessary. An example of an early broncho-pneumonic lesion is shown in fig. 49, where cellular exudate which had undergone caseous change is seen plugging the bronchiole, and there was much peri-bronchial cellular infiltration of small round and large mononuclear cells. Such a lesion was found to be difficult, and, indeed, often impossible to distinguish by the naked-eye from a focus of sub-acute miliary tubercle. For comparison a section from a case with sub-acute miliary lesions is shown in fig. 50, from which it is seen that the lesions were scattered irregularly throughout the lung tissue, and had no relationship to the bronchioles. It should be noted that we are using the term 'sub-acute' in relation to the histological appearance of these foci, around the caseous centres of which small round cells, endothelioid and giant cells were always found, whereas such a reaction was rarely seen in typical acute miliary tubercles. It is of interest to note that it is these sub-acute/

sub-acute miliary lesions, either alone or associated with small tuberculous broncho-pneumonic lesions, which give rise to the typical "snow-storm" appearance of the lungs seen on radiological examination. The typical acute miliary tubercles, unless very abundant, never produce this appearance. In cases with widespread sub-acute miliary tuberculosis of the lungs numerous tubercles in which there were abundant tubercle bacilli were frequently found in the intima of the pulmonary veins (fig. 51). In nearly all the cases where there was secondary extension of the disease through the air passages to other parts of the lungs, the actual primary lesion showed cavitation or early breaking down, either on naked-eye or microscopic examination. Cases with chronic lesions were rare, and were only met with in children over 4 years and 9 months. In 10 cases no other tuberculous lesions, apart from the primary focus, were found in the lung substance, though in 7 of these the pleura was affected, and in all of them the related tracheo-bronchial glands were the seat of tuberculous adenitis.

(2) Bronchi. In 4 cases in which the focus was in the right upper lobe, one of the enlarged caseous superior tracheo-bronchial glands had ulcerated through into the right bronchus. In 3 of these cases the appearance/

appearance of the rupture suggested that it was acute and of recent origin, while in the remaining one there was some evidence of chronic inflammatory reaction around the site of rupture. In this case there was a widespread tuberculous broncho-pneumonic lesion in the lung (fig. 52). The small number of these cases is opposed to the suggestion made by some authors that the primary lung focus is due to rupture into a bronchus of an enlarged caseating tuberculous gland at the hilum of the lung.

(3) Oesophagus. In only one case was perforation of the oesophagus by tuberculous glands noted. In this case an enlarged caseous left para-tracheal gland had become adherent to the anterior wall of the oesophagus and ruptured into that structure, producing an irregular ulcer about the size of a sixpence. In this case already described under a previous heading (fig. 25), there were multiple tuberculous ulcers in the upper part of the small bowel. These were most likely due to the lesion in the oesophagus, as the primary lesion in the lung showed no evidence of breaking down, either on naked-eye or microscopic examination.

(4) Pulmonary blood vessels. In only 2 of the cases was erosion of one of the larger branches of the pulmonary/

pulmonary vessels, with consequent haemorrhage, noted. In both of these there were large caseous-walled cavities near the root of the lung, in one case in the right lower lobe, and in the middle lobe in the other. Both children were over 3 years of age.

(5) Pleura. In 62 cases the primary lung lesion itself was associated with lesions in the pleura, while in 3 others the associated right superior tracheo-bronchial glands which were the seat of tuberculous adenitis had produced pleural lesions. These are summarised in table XVII, from which it will be seen that the pleural changes were at the site of the primary lesion in 44 (71 per cent.) out of the 62 cases, while in 57 (91.9 per cent.) the pleural changes were on the side of the primary lesion. In these cases with chronic pleuritis in both pleural sacs the lesions in the lungs were of a chronic nature, and all occurred in children over 7 years, with the exception of a child of 2 years (see fig. 10) in whom 4 caseous-walled cavities surrounded by fibrous tissue were found in the left upper lobe. As already observed, we found that these pleural changes were useful in helping us to locate the side, and often the actual site of the primary focus. In 3 cases there was a chronic adhesive pleurisy over enlarged caseous right upper tracheo-bronchial glands, related to foci in/

TABLE XVII. **Pleural lesions associated with primary lung foci.**

65 cases	62 cases related to primary focus itself.	57 cases on same side as lung focus.	In area over and around focus.	20 acute tuberculous pleuritis.
				24 chronic adhesive lesions.
			Involving whole side.	12 chronic adhesive lesions.
				1 caseous pleurisy.
		5 cases on both sides.	4 chronic adhesive lesions involving whole of both pleural cavities.	
			1 chronic adhesive pleurisy on both sides. chronic mediastinitis. chronic fibrous pericarditis (atrophy of heart).	
	3 cases related to glands.	3 cases on same side as glands related to lung focus.	3 chronic adhesive lesions in area over caseous right superior tracheo-bronchial glands.	

in the right upper lobe. One of these cases is described in fig. 53. This was the only gland group in connection with which such changes were observed.

(6) Bowel. In 43 of the 102 cases with primary foci numerous tuberculous ulcers were found throughout the bowel. These, however, were most numerous in the small intestine, but occasionally they were also found in the large bowel, and on rare occasions actually in the stomach. In 29 of the cases the primary lesion in the lungs showed definite cavitation, while in the remaining 14 there was evidence of early breaking down of the caseous material in the centre of the foci. In the case of these latter lesions the intestinal ulceration was not so extensive as in the cases with definite cavities in the lungs. The mesenteric glands in relation to these ulcers were caseous and often formed large masses which extended up to the root of the mesentery. This involvement of the intestine, due to sputum infection, was noted throughout all ages, and even in children up to 11 years of age.

(7) Neighbouring glands. In 40 of these cases a direct extension of the glandular disease could be followed either into the lower deep cervical glands or those in the upper part of the abdomen. These cases are summarised in/

TABLE XVIII.

Cases with primary foci in which there was direct extension of the glandular lesion into (1) the lower deep cervical glands; (2) abdominal glands.

(1)

31 cases lower deep cervical glands.

- 24 right
  - 14 cases with primary lesion in right upper lobe.
  - 3 cases with primary lesion in right middle lobe.
  - 6 cases with primary lesion in right lower lobe.
  - 1 case with primary lesions in right middle & lower lobes.
- 7 left
  - 3 cases with primary lesion in left upper lobe.
  - 4 cases with primary lesion in left lower lobe.

(2)

9 cases glands in upper part of abdomen.

- 4 cases with primary focus in right upper lobe.
- 2 cases with primary focus in left upper lobe.
- 2 cases with primary focus in left lower lobe.
- 1 case with primary foci in right upper & left lower lobes.

Tuberculous disease in the cervical and abdominal glands associated with the primary lung focus.

Spread.	Cervical.	Abdominal.
Direct	23	4
Indirect (sputum infection)	3	38
Direct and Indirect	8	5
Total	34	47

in table XVIII. In 2 of the children there was a direct extension of the glandular lesion both upward and downward. The mesenteric glands were involved indirectly through the sputum, causing tuberculous ulceration of the bowel in 43 cases, and these have already been discussed in the preceding paragraph. In the case of the upper cervical glands, these were involved indirectly by the same means in 11 cases, in all of which there were cavities in the lungs. The submaxillary glands in these were the seat of tuberculous lesions, which never extended any distance down into the neck, and in 8 out of these 11 cases tuberculous ulceration was found in the tissues of the pharynx or larynx. In some cases the cervical and abdominal glands were involved by both routes, as is summarised in the second part of table XVIII, from which it is evident that the commonest route by which the cervical glands were involved was by direct spread upward of the tuberculous lesions from the hilum of the lung, while in the case of the abdominal lymph nodes it was indirectly by means of the sputum.

(8) Other lesions. Generalised miliary tuberculosis was found in 88 out of the 102 cases with primary lung lesions. In 71 cases there was cerebral tuberculosis, either meningitis, tuberculomata, or both, while in only 3 cases was tuberculous disease of bone observed (table XXI).

(9)/

(9) The effect of the lung focus on the life of the child.

In all of the 102 cases except one the primary focus had led to a fatal ending, either through generalised miliary tuberculosis, meningitis, or severe pulmonary disease. The one exceptional case, from which a human strain was isolated, occurred in a girl aged 9 years who died of an acute appendicitis, and has already been described in fig. 6.

Cases with double portals of entry of the infection.

In 2 cases, besides the primary lesion in the lung, there were a few tuberculous glands in the mesentery in the ileo-caecal angle. In one of these cases the mesenteric glands showed some fibrous periadenitis. From the focus in the lungs in this case a human strain was isolated and a bovine from the mesenteric. In the other case, a female child aged 7 years, the lung lesion was the seat of early softening of the caseous material, but there was no naked-eye evidence of intestinal ulceration connected with the partly calcified caseous mesenteric glands. From the right inferior tracheo-bronchial glands in this case a human strain was obtained, while from the mesenteric both human and bovine strains were isolated. The presence of the human strain in the latter group of glands was considered/

considered as probably due to infection through the sputum, and accordingly secondary, while the bovine infection was considered primary. The lung lesions in both these cases were more acute than the abdominal, and were regarded as being responsible for death through the occurrence of secondary lesions.

Bacteriological findings in cases with primary lung foci.

The results of the bacteriological investigation in the cases with primary lung lesions are summarised in the following table:-

Source of cultures.	Human strains isolated.	Strains lost.	Total.
Primary focus	32	1	33
Related glands	62	7	69
Total	94	8	102

Thus only human strains were obtained from all these cases. For the most part these cases in which the primary focus was used for isolation were very young children under 2 years, with more acute lesions, in which, as we have already described in the histological section, the bacilli were very numerous. Further discussion of the bacteriological/

bacteriological findings is reserved till the cases which we have classed as doubtful thoracic have been considered.

B. Cases in which no primary lung foci were found; doubtful thoracic cases.

In 21 cases in which the thoracic tissues were considered the seat of the primary infection, no lung focus was discovered. In all of these, however, the morbid anatomical lesions present at post-mortem were most marked in the lungs or thoracic glands, while lesions elsewhere could be accounted for by secondary haematogenous, lymphogenous, or intra-canalicular spread, except in one case where tuberculous adenitis was found in the right superior tracheo-bronchial glands, as well as in one gland in the mesentery at the ileo-caecal angle. A human strain was isolated from the tracheo-bronchial glands in this case, but we failed to isolate any virus from the mesenteric. This was considered, for the purpose of classification, as a double infection, as the lesions did not appear to be anatomically related. The finding of the most advanced tuberculous disease in the thorax in these 21 cases did not absolutely negative the fact that the portal of entry may have been elsewhere, for, as we have already discussed, the virus may have passed through some mucous surface of the body, and then through various lymphatic glands without causing any lesion until the thoracic lymph nodes

were/

were reached. If, however, there were points of similarity between these doubtful cases and those in which primary foci were found, we might be in a position to maintain that the site of infection was primarily in the thorax. For the discussion of these cases we have divided them into three categories:-

(1) Those in which a primary focus might have been found;

(2) Those in which there were severe pulmonary lesions which made the finding of the primary focus impossible;

(3) Those in which it was unlikely that a primary focus existed.

(1) <u>Cases in which a primary focus might have been found</u>.

In the first group there were 9 cases. In 6 of these the tracheo-bronchial glands on the right side were the seat of tuberculous disease, and in 3 those on the left. The glandular distribution of these lesions and the type of infection present are summarised in table XIX, from which it is evident that the distribution of the glandular lesions in these cases did not differ from that observed in cases where primary lung foci were found. Further, it was noted that the glands nearest the lungs were the seat of the most advanced tuberculous disease, while on following the lesion through various glands to those more distant from the lungs it became less marked. It/

TABLE XIX.

Distribution of the glandular lesions in 9 cases in which, though a primary lung focus might have been present, none was found.

9 cases	6 right side.	2 cases	Right broncho-pulmonary " superior tracheo-bronchial. " paratracheal	1 Human 1 Bovine
		2 cases	Right broncho-pulmonary " inferior tracheo-bronchial.	1 Bovine 1 Lost.
		1 case	All the above glands & left paratracheal.	1 Human
		1 case	All the above glands & Right lower cervical Lower mediastinal Coeliac glands	1 Lost
	3 left side.	1 case	Left broncho-pulmonary (intra- and extra-parenchymatous) " superior tracheo-bronchial. " paratracheal. " lower cervical.	1 Human strain.
		2 cases	Left broncho-pulmonary " inferior tracheo-bronchial.	2 Human strains.

It is possible that in these cases the focus may have been so small that it was missed. In the case on the left side in which the intra- and extra-parenchymatous broncho-pulmonary glands were involved this seems to be the only possible explanation. One of the intra-parenchymatous glands may, of course, have been the actual primary lesion in this case, which is described in fig. 54. In 7 of these cases the lungs were the seat of miliary tubercles, while in the other 2 no lesions were noted in the lung substance. When the organisms isolated from these cases were considered we found that bovine strains were present in 2, human in 5, and in the remaining 2 cases there was failure to isolate the infecting virus, as shown in the summary of the distribution of the glandular lesions noted above. As the finding of bovine strains in such lesions is unusual, the description of these cases, with the bacteriological findings, is given in full in figs. 55 and 56.

(2) <u>Cases of severe pulmonary disease which made the finding of the primary focus impossible.</u>

(a) The first group in this division consisted of 3 cases in which there was extensive broncho-pneumonic tuberculosis in both lungs with cavitation. All the subjects were under 9 months of age. The lesions in the tracheo-bronchial glands in these were extensive and acute, as shown/

shown in the case described in fig. 57, and it was not possible to come to any definite conclusion as to which group was first involved. In all these children there was much tuberculous ulceration of the small and large bowel, with involvement of the mesenteric glands. It is possible that in such cases there were multiple acute primary foci which underwent rapid dissolution, and accordingly were not recognisable at autopsy. From all these cases human strains were isolated.

(b) The second group consisted of one child aged 9 years with widespread chronic peri-bronchial tubercle in both lungs. There was chronic adhesive pleurisy on both sides, and a chronic tuberculous pericarditis. The tracheo-bronchial glands on both sides were extensively involved, being the seat of caseation and fibrosis. A human strain was isolated from the right superior tracheo-bronchial glands.

(c) The third group consisted of 5 subjects in whom there was very marked chronic adhesive pleurisy with thickening of both visceral and parietal pleurae. So dense were the adhesions in these cases that it was not possible to separate the two layers of the pleura over the entire surface of the lungs. All the cases were over 2½ years of age, and in 4 of them the most marked tuberculous disease in the/

the tracheo-bronchial glands corresponded to the side of the pleural involvement, as summarised in the following table:-

Cases in which primary foci were not found, but in which there was chronic adhesive pleurisy.

Side	Position of most advanced tuberculous adenitis.	Strain isolated.
Right 2 cases	1 Right broncho-pulmonary " superior tracheo-bronchial	No strain isolated.
	1 Right broncho-pulmonary " inferior tracheo-bronchial	Human strain.
Left 2 cases	2 Left broncho-pulmonary " superior tracheo-bronchial	1 Human. 1 Lost.
Both 1 case	1 Left broncho-pulmonary " superior tracheo-bronchial	No strain isolated.

In 3 of these the lungs were the seat of a sub-acute miliary lesion, while in another, in addition to this, there was early broncho-pneumonic tubercle. In the remaining case no lung lesions were noted. Thus the distribution of the pleural and glandular lesions in these cases was not unlike those with primary foci in which similar lesions were present. The possibility suggests itself that the primary lesion was small, superficial, and/

and concealed by the dense pleural fibrosis, and was accordingly missed at post-mortem. In all of these cases the related tracheo-bronchial glands were the seat of caseation and fibrosis. Only 2 strains (both human) were isolated from the glands in these cases, the failure to isolate the infecting virus in the remaining 3 cases being probably due to the fairly advanced stage of healing present in the lymph glands.

(3) Cases in which the existence of a primary focus was unlikely.

There were 3 cases of this nature, in all of which the most marked tuberculous lesion was in the para-tracheal glands. In all of them the lungs were the seat of abundant miliary and sub-acute miliary tubercles. In 2, from both of which human strains were isolated, only the para-tracheal glands were the seat of tuberculous adenitis, in one case on the right, and in the other on both sides. In neither of these cases was there any evidence of disease in the various groups of tracheo-bronchial glands around the bifurcation of the trachea, or in the cervical or abdominal glands. In the remaining case, described in fig. 58, the most advanced lesion was in one of the left para-tracheal glands a short distance above the bifurcation of the trachea. From this situation the glandular lesion had extended slightly upwards/

upwards and downwards, but became less marked in both directions, the glands in the lower triangle of the neck and the superior tracheo-bronchial glands on the left side showing only a few caseous points. The broncho-pulmonary glands were not, however, the seat of any lesion. From this case a bovine strain was isolated. The distribution of the glandular lesions in these 3 cases was unlike that in any of the others met with in this investigation. The possibility must be admitted that though no disease was found in the upper cervical glands, the infection may have originally come from there. Equally, of course, the infection may have passed through the lung and tracheo-bronchial glands without causing any lesion. Yet another possibility is that the infection may have passed through the mucosa of the trachea or larger bronchi, and so to the glands in direct anatomical relation. That such may occur has been shown by Ranke who has described a case in which he found the primary lesion actually in the mucosa of one of the larger bronchi.

To sum up, then, we found that in 14 of these doubtful thoracic cases there was some similarity in the glandular and pleural lesions to those in which primary lung foci had been found, and in the absence of any lesion showing as advanced tuberculous change in other situations, we considered that the evidence was more in favour of a respiratory/

respiratory portal of entry of the infection than any other. In other 4 children the lung lesions were of a nature (acute broncho-pneumonic tubercle and chronic peri-bronchial tubercle) that could be regarded, in the young child at least, as most likely due to a respiratory source of the infection. In the remaining 3 subjects where the para-tracheal glands were most affected the pathological evidence was inconclusive as regards the primary site of infection. It was impossible, however, to decide where the portal of entry of the infection was likely to have been in these 3 cases, and so, more for the sake of convenience in the description of the bacteriological findings, we have classified them along with the other thoracic cases.

Secondary lesions found in the doubtful thoracic cases.

The lesions in the lungs, pleurae, and bowel have been discussed in connection with the various lesions above, and will not be further mentioned here. There only remain to be summarised the lesions in the neighbouring lymph nodes and general lesions.

(1) Neighbouring lymphatic glands.

In 5 of the cases the neighbouring lymphatic glands were involved. In 4 of these there were small foci/

foci of caseation in the lower deep cervical glands, while in another case a similar lesion was present in the lymph nodes in the upper part of the abdomen. From the thoracic lymph nodes the tuberculous process could be traced direct to the lower cervical glands, and also to those in the upper part of the abdomen, but as the disease approached these limits it was gradually becoming less and less recognisable by the naked-eye.

(2) <u>Other lesions</u>.

Generalised miliary tubercle was noted in 15 of the cases, and in 16 there was tuberculous meningitis p 184 (table XXI). In only 2 out of these 21 cases was the tuberculous disease not the cause of death. In both of these only small caseous areas were found in two of the tracheo-bronchial glands at post-mortem. One was a child aged 5 years who died as the result of a street accident and from whom a human strain was isolated, and the other was a child aged 2 years and 3 months who died from acute peritonitis. From the latter case no organisms were isolated. In this group, as in the cases showing primary lung lesions, the finding at post-mortem of tuberculous disease which did not cause death was very rare.

<u>Bacteriological findings in these doubtful thoracic cases</u>.

This/

This has been mentioned in the various sections dealing with the lesions, and is summarised in the following table:-

Source of Cultures.	Human.	Bovine.	Lost.	Total.
Tracheo-bronchial glands	11	2	5	18
Para-tracheal glands	2	1	-	3
Total	13	3	5	21

The bacteriological findings will be discussed more fully later.

The age distribution of cases in which the infection was considered as primary in the thoracic tissues.

In table XX the age distribution of the cases has been given for yearly age-groups. Both cases with and without primary lung foci were most frequent in the first year of life. Of the total cases with the primary site of infection in the thoracic tissues, the greatest number was found in the first year, although such cases were very rare in the first 3 months, only 4 having been/

TABLE XX. The age distribution of the cases in which the site of the primary infection was considered thoracic and their relation to the cause of death.

Type of case.	Result of lesion.	0-1 yr	1-2 yrs	2-3 yrs	3-4 yrs	4-5 yrs	5-6 yrs	6-7 yrs	7-8 yrs	8-9 yrs	9-10 yrs	10-11 yrs	11-12 yrs	Total.	
102 cases with primary lung foci.	Cause of death.	44	24	12*	1	4	2	5*	1	3	1	3	1	101	102
	Not cause of death.	-	-	-	-	-	-	-	-	1	-	-	-	1	
21 cases with no primary lung foci.	Cause of death.	5	3	2*	3	1	2	-	-	2	-	1	-	19	21
	Not cause of death.	-	-	1	-	1	-	-	-	-	-	-	-	2	
Total primary thoracic cases = 123.		49	27	15	4	6	4	5	1	6	1	4	1	123	
Percentage of total cases with tuberculous lesions (table IX).		65.3	50.9	55.6	41.2			66.7			66.7			56.9	

Lesions cause of death = 120 (97.6 per cent.)
Lesions not cause of death = 3 (2.4 per cent.)

*There was one case in each of these three age-groups in which a separate primary abdominal lesion not the cause of death was present. (See table XXV).

been found in the present investigation, while there were 14 in the second 3 months, 19 in the third, and 12 in the fourth. After the first year there was a steady fall to the third year, from which age the numbers became too small for comparison by yearly groups, though it is to be noted that in each of the age groups after the third the number of cases was much smaller than that found in that year. When the number of cases with thoracic lesions was expressed as a percentage of the total 216 cases in which tuberculous lesions were found at autopsy, and arranged in similar age-groups as in table IX, it was observed that the lesions associated with the respiratory system constituted the majority of the tuberculous lesions in the first year and also from the seventh to the twelfth year, while in the second and third years they were little more than half, and less than this in the fourth to the sixth. That is, the thoracic types of lesions were found to be most common in early infancy and also in later childhood. Further, on comparing these percentages with those obtained for abdominal lesions p. 204 (table XXV) it was noted that in all the age-groups the thoracic lesions were the commoner. Only a very small percentage (2.4) of cases with the primary site of infection in the thorax did not cause death, and all these were in children over 2 years of age, while in 97.6 per cent. the tuberculous disease was/

was either the direct or indirect cause of death. This has also been noted by Shennan (1914) and McGregor (1925) in their investigations into the frequency of tuberculous disease in children at post-mortem in Edinburgh. Further, Findlay (1926) from clinical and radiological studies has also commented on the rarity of non-fatal tuberculous thoracic lesions. On the other hand, these results are at variance with those of many continental and American workers who frequently found tuberculous lesions in the thoracic tissues which had not been the cause of death.

Comparison of secondary lesions in thoracic cases with and without primary foci, and with those occurring in abdominal cases.

When the percentagesof secondary lesions in the cases with and without primary lung foci were compared (table XXI), there was a suggestion that the spread of the tuberculous lymphadenitis, the occurrence of pleural lesions, generalised miliary tuberculosis, secondary lesions in bone and bowel, and the percentage of fatal cases were all more frequent in the cases with a primary lesion in the lungs than in those without. The numbers available, however, particularly in the cases with no primary lung lesion, were much too small to warrant a definite statement on these points. On comparing the percentage/

TABLE XXI. The secondary lesions in cases where primary infection was considered to be thoracic.

Type of case.	Total cases.	Spread to other glands. Cervical. Direct	Cervical. Indirect	Abdominal. Direct	Abdominal. Indirect	Pleural lesions	Gen. Mil. T.B.	Cerebral T.B.	Secondary lesions in Bones.	Secondary lesions in Bowel.	Fatal cases	Lesions not cause of death.
Cases with primary lung foci.	102	31	11	9	43	65	88	71	3	43	101	1
	Percentage.	30.4	10.8	8.8	42.2	63.7	86.3	69.6	2.9	42.2	99.	1
Cases without lung foci.	21	4	-	1	-	6	15	16	-	3	19	2
	Percentage.	19.	-	4.8	-	28.6	71.4	76.2	-	14.3	90.5	9.5
Total thoracic cases.	123	35	11	10	43	71	103	87	3	46	120	3
	Percentage.	Total direct spread out of thorax = 45 cases.			36.6	57.7	83.7	70.7	2.4	37.4	97.6	2.4
Abdominal cases for comparison.	Percentage	Spread out of abdomen			14.3	-	46.4	45.2	4.8	-	76.2	23.8

percentage of cases with secondary lesions found in the thoracic group with those in the abdominal it was noted that the spread of the glandular lesion out of the region (thorax or abdomen) in which the primary site of infection was present was commoner in the case of thoracic lesions. The relative frequency of generalised miliary tuberculosis and cerebral tubercle was also greater in thoracic cases. On the other hand, a much larger percentage of lesions not causing death was found in those cases considered primarily abdominal. This may be partly due to the anatomical differences in the two types of tissue in which the lesions were situated. For example, a tuberculous lesion in a vascular organ like the lung is more likely to be followed by generalisation than one in the bowel or mesenteric glands. In addition, the tuberculous thoracic glands are much more intimately connected with the vascular system than the abdominal as the efferents of the former join with others to form the right and left lymphatic trunks which drain directly into the great veins. As we shall point out, however, when we consider the bacteriological findings, the difference in the type of infection found in the majority of the two classes of cases may have been also partly responsible for the greater frequency of secondary lesions in thoracic cases as compared with abdominal.

TABLE XXII.

TABLE XXII.

Age distribution, etc., of 120 human strains and 45 bovine strains causing death.
1,300 consecutive autopsies.

	0 to 6 mths	6+ to 12 mths.	1+ to 2 yrs.	2+ to 3 yrs.	3+ to 5 yrs.	5+ to 12 yrs.	Total.
Total autopsies	622	234	177	60	80	127	1,300
Total cases dead of tubercle.	27	45	51	24	20	25	192
Pr. Thoracic lesions	18	31	27	13(2x2)	10	21(1x2)	120(3x2)
Types isolated (Human	18*	28*	22	12(2x2)	8	17(1x2)**	105(3x2)) 97.2 per cent. Hum.
Types isolated (Bovine	-	1	1	1	-	-	3) 2.8 per cent. Bov.
Lost or dead	-	2	4	-	2	4	12
Pr. Abdominal lesions	8	14	23	9	6	4	64
Types isolated (Human	1	2	5	1	-	1	10) 19.6 per cent. Human.
Types isolated (Bovine	5	8	16*	5	4	3*	41) 80.4 per cent. Bovine.
Lost or dead	2	4	2	3	2	-	13
Pr. Elsewhere	1	-	1	2	4	-	8
Types isolated (Human	1	-	1	1	2	-	5) 83.3 per cent. Human.
Types isolated (Bovine	-	-	-	-	1	-	1) 16.7 per cent. Bovine.
Lost or dead	-	-	-	1	1	-	2
Per cent. Human	80	76.9	62.2	70	66.7	85.7	72.7
Per cent. Bovine	20	23.1	37.8	30	33.3	14.3	27.3

*=In thoracic lesions that 4 similar strains were obtained from secondary abdominal lesions.

*=In abdominal lesions that 2 similar strains were obtained from secondary thoracic lesions.

These 6 strains derived from secondary lesions not included in this Table.

(1x2) = One case with double primary foci.

(2x2) = 2 cases " " " " , etc.

In these 3 cases the thoracic focus responsible for death has been included in the above Table, the abdominal focus being included in Table XXIII.

Pr. Thoracic Lesions = Most advanced lesions in thoracic glands or lung.

Pr. Abdominal Lesions = " " " " bowel, abdominal glands, or peritoneum.

Pr. Elsewhere = Primary lesion not in thorax or abdomen, e.g., cervical glands, or unknown.

Lost or dead = Failure to isolate the infecting organisms either on account of technical difficulties or because the tubercle bacilli in the material used were dead.

TABLE XXIII.

Age distribution, etc., of 4 human and 14 bovine strains from tuberculous lesion not causing death.
1,300 consecutive autopsies. (192 cases dead of tubercle, **Table XXII**).

	2 yrs.	2+ to 5 yrs.	5+ to 12 yrs.	Total.
Total autopsies.	1033	140	127	1,300
Non-fatal tuberculous lesions.	5	11	11	27 (3x2)
Pr. Thoracic lesions	-	2	1	3
Types isolated (Human	-	1	1	2
Types isolated (Bovine	-	-	-	-
Lost or dead	-	1	-	1

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	2 yrs.	2+ to 5 yrs.	5+ to 12 yrs.	Total.	
Total autopsies.	1033	140	127	1,300	
Non-fatal tuberculous lesions.	5	11	11	27 (3x2)	
Pr. Thoracic lesions	-	2	1	3	
Types isolated (Human	-	1	1	2	
Types isolated (Bovine	-	-	-	-	
Lost or dead	-	1	-	1	
Pr. Abdominal lesions	3	7(2x2)	10(1x2)	20(3x2)	
Types isolated (Human	-	1	1	2	) 13.3 per cent. Human.
Types isolated (Bovine	3	4(1x2)	6 (1x2) *	13(2x2)	) 86.7 per cent. Bovine.
Lost or dead	-	2(1x2)	3	5(1x2)	
Pr. Elsewhere	2	2	-	4	
Types isolated (Human	-	-	-	-	
Types isolated (Bovine	1	-	-	1	
Lost or dead	1	2	-	3	
Per cent. Human	-	33.3	25	22.2	
Per cent. Bovine	100	66.7	75	77.8	

* Mixed strain due to sputum infection.
Human type isolated from thoracic lesion.

(1x2) = one case with double primary foci.
(2x2) = two cases " " " etc.

In these 3 cases the thoracic focus (table XXII) was considered responsible for death.

included with those found in primary thoracic infections. Of the 107 children infected with the human type, 105 (98.1 per cent.) died as a direct result of the infection, while 2 (1.9 per cent.) died from other causes, small tuberculous foci being found in the thorax at post-mortem. All 3 of the bovine infections were fatal. When the 108 fatal cases of tuberculosis with a respiratory portal of entry, from which the infecting organisms were isolated, were considered, 97.2 per cent. were found to be human infections, and 2.8 per cent. bovine. In the Local Government Board's (1914) investigation in London children aged 2 to 10 years 64 out of 65 cases of thoracic tuberculosis were found to be due to infection with the human virus, the remaining case being a bovine infection in an apparently normal bronchial gland.

Infections with human virus.

It was found that there was a family history of tuberculosis in 32 (29.9 per cent.) out of the 107 cases in which the human type of bacillus was isolated, and in 25 of these it was in a member of the child's own family. While the number of histories of exposure to infection was low, being just less than one-third, yet the family histories given by parents are often unsatisfactory, and in this series the number of negative family histories would/

would have been greater if my clinical colleagues had not particularly investigated such complaints as chronic bronchitis, anaemia, weakness, etc., on the part of the parents, who, when examined clinically and radiologically, were found in some instances to be suffering from pulmonary tuberculosis. Chandler and Preston (1925) in a clinical study of pulmonary tuberculosis in 89 children obtained a positive family history in 49 (56 per cent.) of the cases. It is widely recognised that the respiratory apparatus of the young child is highly susceptible to infection by the human type of tubercle bacillus, with the production of a progressive and fatal disease. This is borne out by the fact that in the 105 fatal cases in our series in which this type of infection was present 80 (76.2 per cent.) were under 3 years of age. It would seem that once the infection produces recognisable lesions in the respiratory system in the child under 3 years there is little hope of cure, though in older children, and in the adult, the position is different. In this investigation it was observed that death from the human type of infection in the thoracic organs was commoner in the first half of the year, when out of the 105 fatal cases 63 deaths occurred as against 39 in the second half. This is probably due to the fact that children are confined more to the house during the colder months of the year, and in consequence in congested/

congested areas are more liable to be infected. Also, the catarrhal ailments of the respiratory system are commoner in the later and earlier months of the year, and these may prepare the soil for infection with the tubercle bacillus which, having gained a foothold, causes a progressive lesion which kills the child a few months later. That the congested conditions of city life, with the consequent greater chances of infection, are further predisposing causes was demonstrated when a comparison was made of the number of city and country children dying from tuberculosis in whom the portal of entry was considered to be in the respiratory system. In the cases under discussion 91 (86.7 per cent.) were city children and 14 (13.3 per cent.) country, and while these figures are open to the criticism that probably more city children were admitted to the hospital, yet the proportion of city to country children admitted during the period of this research was 23 of the former to 13 of the latter, which made the incidence of the primary respiratory infection due to the human virus very much commoner in the city child. By some pediatricians pulmonary tuberculosis is regarded as very liable to occur shortly after an attack of whooping cough or measles. In the present series out of the 107 children with human infections in the thorax a history of one or other of these illnesses within 6 months before admission was/

was only noted in 23. While a statement on prophylaxis might be considered out of place from the results of an investigation such as this one, yet it is evident from our findings that the infant and young child should be rigorously protected against any risk of infection from human sources, though admittedly the control and the hygienic education of the "open" carriers of the human type of tubercle bacillus are much more difficult than the destruction of the less fatal milk-borne bovine virus.

Infections with bovine virus.

The 3 cases in which a bovine type of virus was found are of interest in that only three other such cases, as far as we know, have been reported in this country - Griffith (1919 and 1930 (1)) - though Eastwood and F. Griffith (1914 (3)) found a bovine strain in a normal bronchial gland of a child who died from anaemia, and in whom no evidence of tuberculous disease was found at autopsy. On the continent 3 other cases of apparently primary infection with the bovine virus in the thoracic tissues have, as far as we can find, been described, 2 by Gaffky (1907) in the bronchial glands, in both of which he was unable, after repeated attempts, to isolate in culture the infecting virus from the guinea-pig lesions, and he concluded, partly on/

on this account and partly on the morphological appearances, that they were of bovine type. The third case was reported by Rothe (1911) who isolated, in a case of caseous pulmonary tuberculosis with caseating tracheo-bronchial glands, a bovine strain from the diseased lymphatic glands, and also from one out of 4 apparently normal mesenteric glands. The three cases in which we found the bovine type of virus have been fully described above (figs. 55, 56, and 58).

(2) Cases in which the primary site of the infection was considered to be in the abdomen.

In 84 cases or 38.9 per cent. of the total cases showing tuberculous lesions, the pathological evidence was in favour of the primary site of infection being in the abdomen. These cases have been divided into:-

A. Those in which ulcers were found in the bowel;

B. Those without any naked-eye evidence of ulceration.

  (a) Cases in which the glands showed evidence of healing;
  (b) Cases where there was no healing.

A. Cases with intestinal ulceration.

Number, type, and site of ulcers.

In 15 cases naked-eye evidence of intestinal ulceration was noted. The age groups into which these fall are indicated in table p.204 XXV, from which it is seen that/

that they all occurred in children under 5 years of age, just more than half, 8 out of 15, being noted in the first year of life, and thereafter gradually diminishing in frequency. In all of these cases the tuberculous lesion in the bowel had been responsible for death, either on account of tuberculous peritonitis, meningitis, or generalised miliary tuberculosis. Only in 2 of the cases were there more than 5 ulcers. In 9 cases between 2 and 5 were found, while 4 cases had one each. In some of the cases one ulcer appeared to be more chronic and older than the others which were situated at a lower level in the bowel, and might have been due to secondary infection from the primary ulcerated area. The ulcers were for the most part chronic in nature, though in a few cases they were more sub-acute, but the multiple acute ulcers seen in sputum infections in connection with thoracic cases were never met with. The distribution of these ulcers was as follows:-

15 cases with ulcers.	12 lower end of ileum.
	1 upper part of ileum.
	2 lower end of ileum and caecum.

Thus these ulcers indicating the primary site of infection in the abdominal cases were found most often at the lower end of the small bowel, and were unlike the/

the more acute sputum ulcers noted in cases of pulmonary tuberculosis with cavitation which were observed throughout the whole of the small intestine as well as occasionally in the large. The age of the child had no influence upon the appearance, number, type, or situation of these ulcers.

<u>Secondary lesions</u>.

The mesenteric glands in relation to the ulcers were always enlarged and caseous. The distribution of the tuberculous adenitis in relation to the ulcers was as follows:-

<table>
<tr><td rowspan="4">15 cases with ulcers</td><td colspan="3">1 juxta-intestinal and intermediate mesenteric glands in relation to ulcer segment only involved.</td></tr>
<tr><td rowspan="3">14 widespread involvement right up to terminal mesenteric glands around origin of superior mesenteric artery.</td><td rowspan="3">Of these 6</td><td>2 spread to glands in upper part of abdomen (those around coeliac axis and pancreas, glands in hilum of liver and spleen, lumbar glands).</td></tr>
<tr><td>1 in addition to the above spread through diaphragm to nodes in lower mediastinum.</td></tr>
<tr><td>3 in addition to both the above, spread to glands in upper mediastinum, with involvement of tracheo-bronchial nodes.</td></tr>
</table>

It was occasionally noted that the small juxta-intestinal glands in direct anatomical relation to the ulcers might be free of any naked-eye evidence of tubercle, while the intermediate/

intermediate glands in the mesentery were much enlarged and caseous. In nearly all of the cases the glands, in addition to being caseous, showed breaking-down. In none of them was there any evidence of an attempt at healing, either in the form of fibrosis or calcification.

It will be seen from the scheme showing the spread of the glandular lesions that in 6 out of the 15 cases there was extension of the tuberculous adenitis from the glands at the root of the mesentery upwards, and in 4 of these, spread into the thorax had occurred. There was a diminution, however, in the degree of tuberculous adenitis as it passed upwards through the various groups of lymphatic glands. The 4 cases with spread of the tuberculous disease into the thoracic lymph nodes were all met with in children over 2 years of age, and were all bovine infections. In the 3 cases where the tracheo-bronchial glands were involved, these had been picked out in a very irregular manner which did not suggest any definite anatomical relationship with the various lobes of the lungs as was the case where we found primary lung lesions. In all of these cases, however, we searched for a primary lung focus, but did not find any.

In 8 out of the 15 cases there was a typical tuberculous peritonitis present. In 4 of these the lesions were more of a formative than an exudative nature, numerous plaque/

plaque-like areas of caseation being situated over the visceral and parietal peritoneum. Here again no relation to type of infection or age could be demonstrated. There was generalised miliary tubercle in 11 of the cases, the lungs being involved in all of these, tuberculous meningitis in 10, and in one case there was tuberculous disease of the middle ear and mastoid region (table XXVI). None of these secondary lesions had any constant relationship to age or type of the infection. Thus in all these cases with intestinal ulceration except one the glandular lesions were severe and extensive, there being a spread of the lesion throughout all the glands of the mesentery up to the root in 14 out of the 15 cases. Also, tuberculous peritonitis was met with in just more than half the cases, while generalised miliary tuberculosis and cerebral tuberculosis were present in the majority.

The type of feeding which these children with intestinal ulceration received is interesting, and is summarised below:-

<u>0-1 year</u>.

8 children fed with cow's milk (4 bovine infections.
(1 human infection.
(3 strains lost.

<u>0-2 years</u>.

1 child breast-fed till death at 14 months	-	Human infection.
1 " " for 10 months, then Grade A milk till death at 22 months.	-	Bovine infection.
1 " fed with cow's milk from birth	-	Bovine infection.

We have only included in the above, children up to 2 years of age, as after that age the food taken by children becomes much too general to have any significant bearing upon the subject under discussion. Indeed this applies to some extent to children between 1 and 2 years. The results are, however, suggestive that bottle-fed babies are more liable to tuberculous intestinal ulceration due to the bovine type of infection than the breast-fed. The case which was breast-fed for 10 months, and received Grade A milk thereafter is interesting in view of the finding of a bovine type of infection. Park (1927) has also recovered bovine bacilli from tuberculous cervical glands of 2 children fed with Grade A milk.

B. Cases with no naked-eye evidence of intestinal ulceration.

In the remaining 69 cases the abdominal lymph nodes were the seat of the most advanced tuberculous lesions, but there was no naked-eye evidence of intestinal ulceration. These cases have been divided into (a) those with evidence of healing, and (b) those where no such evidence was observed.

(a) Those with evidence of healing. In 11 cases there were signs of healing as follows:-

11 cases — 5 showed fibrosis - all bovine infections.

6 showed deposit of calcium salts.
(4 bovine infections.
(1 human infection.
(1 in which strain was lost.

From this it will be seen that all the cases showing fibrosis, and the majority of those with calcification, were due to infection with the bovine virus, so that it would appear that the lesions caused by this type of bacillus tend to heal more readily than those due to human strains. In the cases with peri-glandular fibrosis the children were all over one year, while in those showing signs of calcification the youngest was 2 years and 11 months. The naked-eye evidence of the deposit of calcium salts was thus noted at an earlier age in the case of the mesenteric glands than in the case of the tracheo-bronchial. From table XXIV it will be seen that the glandular lesions in these cases were very limited. In none of the cases was tuberculous peritonitis present. Generalised miliary tuberculosis was noted in 2 of the cases, and cerebral tuberculosis in 3. In 8 of these children with evidence of healing in the mesenteric glands the tuberculous lesion was found accidentally at post-mortem, and had not been the cause of death (table XXVI).

(b)/

TABLE XXIV. Spread of the tuberculous adenitis in 69 cases with caseous mesenteric glands which were not related to any recognisable ulcerative process in the bowel.

69 cases	(a) Cases with evidence of healing.			
	11 cases	9 tuberculous adenitis limited to one sector of mesentery and not extending to root.		9 Bov.
		1 diffuse and extending to root		1 Hum.
		1 " " " " " and slight in coeliac glands		1 Lost.
	(b) Cases with no evidence of healing.			
	23 cases	Tuberculous adenitis limited to one sector of mesentery and not extending to glands at root.	20 related to lower end of ileum.	11 Bov. 4 Hum. 5 Lost.
			3 related to middle of small bowel.	2 Bov. 1 Hum.
		Diffuse tuberculous adenitis	7 Bovine	

TABLE XXIV.

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69 cases	(a) Cases with evidence of healing.			
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		1 diffuse and extending to root		1 Hum.
		1 " " " " " and slight in coeliac glands		1 Lost.
	(b) Cases with no evidence of healing.			
	23 cases	Tuberculous adenitis limited to one sector of mesentery and not extending to glands at root.	20 related to lower end of ileum.	11 Bov. 4 Hum. 5 Lost.
			3 related to middle of small bowel.	2 Bov. 1 Hum.
	14 cases	Diffuse tuberculous adenitis of mesenteric glands and extending to glands at root.	7 Bovine. 1 Human. 6 Lost.	
	13 cases	All abdominal glands involved.	10 Bovine. 2 Human. 1 Lost.	
	8 cases	All abdominal and some thoracic involved.	4 to glands in lower mediastinum only.	2 Bov. 1 Hum. 1 Lost.
			4 to glands in upper mediastinum. (1 case per internal mammary glands).	4 Bov.

Bov. = Bovine strains.

Hum. = Human strains.

Lost = Failure to isolate infecting virus.

(b) Those with no evidence of healing.

No evidence of healing was noted in the remaining 58 cases in which the glands were the seat of caseation. In many of these cases there was extensive softening of the caseous material in the glands. In table XXIV the distribution of the glandular lesion is summarised, from which it will be seen that in 23 out of the 58 cases tuberculous adenitis affected only a few of the mesenteric glands, and did not extend to the glands at the root of the mesentery. The glands in relation to the lower end of the ileum were found to be most commonly affected in these cases. In the remaining 35 children the glandular disease had involved the mesenteric nodes right up to the root of the mesentery, while in 21 of them, as will be seen from the table, there had been further extension. In one of the cases where the glands in the upper mediastinum were involved the glandular lesion had spread from the abdomen along the internal mammary glands instead of through the nodes in the lower mediastinum. In all of these various groups the bovine infections were commoner than the human. The age of the child was found to have no constant relationship to the extent of the glandular lesions. In table XXVI the secondary lesions found in these 58 cases of mesenteric/

mesenteric gland tubercle without evidence of healing have been summarised. In 20 of the 58 cases tuberculous peritonitis was present, and the ages of the children with this complication varied from 4½ months to 12 years. Generalised miliary tubercle was noted in 26 of the cases, and in 25 cerebral tuberculosis. The ages of the subjects had no effect on the occurrence of these secondary lesions.

Bacteriological findings in the abdominal cases.

In the following table the results of the bacteriological investigation in relation to the various abdominal lesions described above are summarised.

Type of Case.	Total Cases.	Human	Bovine	Lost
With intestinal ulcers	15	2	9	4
No ulcers — Glands healing	11	1	9	1
No ulcers — Glands caseous. No healing.	58	9	36	13
Total	84	12	54	18

Further discussion on these findings is given more fully later.

The age distribution of cases in which the primary site of infection was considered to be in the abdomen.

The age distribution of the cases with primary lesions in the abdomen is summarised in table XXV, from which it is evident that the greatest incidence of cases was found in the first two years of life, the number being slightly higher in the second than in the first year in which 1 case was found in the first three months, 7 in the second, 6 in the third, and 9 in the fourth. In the third year the number had fallen to practically half of what it was in the second year. After the third year the numbers became too small to have any real significance. It is to be noted, however, that the frequency of primary abdominal lesions in the later years was very much lower than in the first two years of life. When the number of cases with primary abdominal lesions was expressed as a percentage of the total 216 cases showing tuberculous disease post-mortem, and arranged in similar age groups as in table IX, it was found that through all the age groups the abdominal lesions always composed less than 50 per cent. of the total, only approaching this percentage in the second year where they constituted 47.2 per cent. of the total tuberculous lesions. This, as we have already noted, may be due to the child picking up the infection/

TABLE XXV. **The results produced and the age distribution of the cases in which the site of the primary infection was considered abdominal.**

Type of lesion.	Morbid changes in mesenteric glands.	Result of lesion.	0-1 yr	1-2 yrs	2-3 yrs	3-4 yrs	4-5 yrs	5-6 yrs	6-7 yrs	7-8 yrs	8-9 yrs	9-10 yrs	10-11 yrs	11-12 yrs	Total.	
15 cases with intestinal ulcers.	No healing.	Cause of death.	8	3	2	1	1	-	-	-	-	-	-	-	15	15
		Not cause of death.	-	-	-	-	-	-	-	-	-	-	-	-	0	
69 cases without intestinal ulcers.	11 with healing in glands.	Cause of death.	-	1	1	1	-	-	-	-	-	-	-	-	3	11
		Not cause of death.	1	-	1*	1	-	1	3*	1	-	-	-	-	8	
	58 with no healing in glands.	Cause of death.	14	19	6	2	1	1	1	-	-	-	1	1	46	58
		Not cause of death.	-	2	2*	-	3	2	1	-	1	-	-	1	12	
Total primary abdominal cases = 84			23	25	12	5	5	4	5	1	1	-	1	2	84	
Percentage of total cases with tuberculous lesions (table IX)			30.7	47.2	44.4	41.2			38.9			33.3			38.9	

Lesions cause of death = 64 (76.2 per cent.)
Lesions not cause of death = 20 (23.8 per cent.)

*There was one case in each of these three age-groups in which there was a separate primary thoracic lesion which was responsible for death. (See table XX).

infection from the floors, etc., and also to milk-borne infection. It was at this period, as shall be described later, that the greatest number of human infections in the mesenteric glands were noted as well as the greatest percentage of fatal bovine infections. When these percentages were compared with those obtained for thoracic cases (table XX) it was noted that through all the age groups the thoracic percentage was higher; the abdominal only approached it during the second year, and also from the fourth to sixth years.

The frequency of lesions which were met with accidentally at post-mortem and not actually the cause of death was found to be greatest after the first year of life. Even although the number of these lesions not causing death was small throughout the various age groups, yet in nearly every age group some such cases were present. This was in marked contrast to what was noted in the case of tuberculous lesions not causing death found in the thorax, which, as we have already shown, were very infrequent, occurring in only 3 (2.4 per cent.) of the 123 thoracic cases. On the other hand, 20 (23.8 per cent.) out of the 84 lesions considered as primary abdominal infections were not the cause of death. As we shall point out in the section dealing with the bacteriological findings in these cases, this may have been due to a difference in the type of infection in these two classes

of/

of cases. There is to be considered, also, the difference in the anatomical site of the two infections, and this has already been discussed in the section dealing with the thoracic cases.

<u>The secondary lesions in cases with the primary site of infection in the abdomen</u>.

In table XXVI the secondary lesions found in the various classes of abdominal cases are summarised. It is seen from the table that in the cases with evidence of intestinal ulceration there was more extensive spread of the tuberculous lymphadenitis, and the frequency of tuberculous peritonitis, generalised miliary tuberculosis and cerebral tuberculosis was greater than in those cases showing no evidence of ulceration. Further, all the cases with intestinal ulceration ended fatally, while this occurred in only 71 per cent. of those without ulcers. It was also noted that the spread of glandular lesions was more extensive, and tuberculous peritonitis, generalised miliary tuberculosis and cerebral tuberculosis were more frequent in those cases where there were no signs of fibrosis or calcification in the tuberculous lesion in the mesenteric glands as compared with those in which evidence of healing was present. Further, the percentage/

TABLE XXVI. The secondary lesions in cases where the primary site of infection was considered to be in the abdomen.

Type of case.	Total cases and Percentage.	Spread of glandular lesion from mesenteric glands. To other abdominal glands.	Spread of glandular lesion from mesenteric glands. To thoracic glands.	Tuberculous peritonitis.	Generalised miliary tuberculosis.	Cerebral tuberculosis.	Secondary lesions in bones.	Fatal cases.	Lesions not cause of death.
Cases with intestinal ulcers.	15 Percentage.	6 40	4 26.7	8 53.3	11 73.3	10 66.7	1 6.7	15 100	- -
Cases without ulcers. (a) Signs of healing.	11 Percentage.	1 9.1	0 0	0 0	2 18.2	3 27.3	1 9.1	3 27.3	8 72.7
(b) No healing.	58 Percentage.	21 36.2	8 13.8	20 34.5	26 44.8	25 43.1	2 3.4	46 79.3	12 20.7
Total without ulcers.	69 Percentage.	22 31.9	8 11.6	20 29	28 40.6	28 40.6	3 4.3	49 71	20 29
Total abdominal cases.	84 Percentage.	28 Spread out of abdomen = 14.3	12	28 33.3	39 46.4	38 45.2	4 4.8	64 76.2	20 23.8
Thoracic cases for comparison.	Percentage.	Direct spread out of thorax = 36.6		-	83.7	70.7	2.4	97.6	2.4

percentage of lesions found accidentally at post-mortem and not the cause of death in cases showing signs of healing was very high (72.7 per cent.), while in those cases showing no evidence of arrest of the disease it was much lower (20.7 per cent.). This was what we would have expected as the healing process confined the infection to a limited area. There was relatively fewer secondary lesions (generalised miliary tuberculosis, cerebral tuberculosis) and less glandular spread in the cases of primary abdominal lesions as compared with those in which the primary site of the infection was in the thorax. The reasons for this have already been discussed in the section dealing with primary thoracic lesions.

Bacteriological findings in those cases in which the primary site of infection was considered abdominal.

Under this heading the different types of virus found in the 84 cases, the pathology of which has just been discussed, are fully considered. The material used for isolation was mesenteric or retroperitoneal glands, or portions of diseased omentum. Failure to isolate the infecting organism occurred in 18 cases, while from the remaining 66 children the bovine type was isolated from 54/

54 (81.8 per cent.), and the human from 12 (18.2 per cent.), as shown in tables XXII and XXIII. Other 4 human strains were isolated from the abdominal lymphatic glands, but in each of these cases there was evidence that the lesions in these glands were secondary to a sputum infection from the breaking-down of the lung lesions, and accordingly these strains have not been included under this heading. Of these infected with the bovine type, 41 (75.9 per cent.) died directly as a result of this infection, while in the remaining 13 (24.1 per cent.) a tuberculous lesion was found in the abdominal glands post-mortem, the children having died of other disease. Included in this group are 2 cases with double portals of infection,- thoracic and abdominal - the tuberculous lesions in the thorax, from which human viruses were isolated, being probably responsible for death. In the case of the 12 children from whom the human type was isolated, 10 (83.3 per cent.) died of tuberculosis, while 2 (16.7 per cent.) died of other diseases. When all the fatal cases of abdominal tuberculosis were considered together, 41 (80.4 per cent.) were bovine, and 10 (19.6 per cent.) human infections, while of the abdominal lesions not causing death 13 (86.7 per cent.) were bovine, and 2 (13.3 per cent.) human. In the investigation already quoted in London children by the Local Government Board/

Board (1914) 18 (78.3 per cent.) out of 23 cases of alimentary tuberculosis were due to infections with the bovine type of virus, so that in the case of abdominal tuberculosis this type of infection would appear to be slightly commoner in Scottish children.

It was found in these abdominal cases that country children were slightly more frequently infected with the bovine type than those in the Glasgow area, in the former the proportion of human to bovine infections being 3 human (12 per cent.) to 22 bovine (88 per cent.), while in the latter 9 were human (22 per cent.) and 32 bovine (78 per cent.). This may be due to the bulk of the city milk, as already pointed out, being pasteurised, and under strict sanitary and veterinary control, whereas in the rural districts the milk is either sold direct by the farmers themselves or to small dairies which have not the extensive and efficient pasteurising plant found in the large milk distributing centres in the city area.

That breast-feeding had a beneficial effect in reducing the number of both types of infection, though particularly the bovine, was observed when we considered children with foci of tuberculosis in the abdomen up to 1 year of age who were breast-fed for about 9 months. In such children there were only 1 human and 1 bovine infection, whereas in the corresponding bottle-fed babies there/

there were 3 human and 12 bovine infections.

The sex of the child appeared to have little influence on the type of infection, as of the male children 18.9 per cent. were infected with human strains and 81.1 per cent. with bovine, while of the females 17.2 per cent. were infected with the former and 82.8 per cent. with the latter type of virus.

The occurrence of measles, whooping cough or scarlet fever in the 6 months prior to admission was noted only in one of the human infected cases and in 8 of the bovine, while a history of tuberculosis in a member of the patient's own family was obtained in 5 of the 12 cases from which the human virus was isolated, while only 4 of the 54 bovine infected cases gave such a history. So it would appear that a tuberculous family history is a predisposing factor in the case of an abdominal tuberculous lesion caused by the human type of bacillus. It was noted also that the greatest incidence of this type of infection in the present series occurred during, and immediately after the period when the child usually commences to crawl and walk, 7 out of the 12 human infections being found in children aged from 9 to 18 months. At this period it is likely that young children in the poorer class districts get their hands contaminated from floors or ground on which there is tuberculous expectoration which/

which is conveyed to the mouth and swallowed. In connection with the finding of these human strains in primary abdominal lesions in children at the period when they start to move about for themselves, and also in those with tuberculous family histories, the observations of Scott (1921) are of much importance. This worker noted that, in the case of Chinese children up to 10 years of age who do not, as a rule, take milk as a food, out of 225 cases with tuberculous lesions 34 (15.1 per cent.) showed evidence post-mortem that the portal of entry of the infection was through the alimentary system. This he partly attributed to the widespread habit of spitting amongst the native people of the East, so that young children became infected from expectoration found on the floors of their dwellings.

As in the case of thoracic tuberculosis, a similar seasonal fluctuation was found in the death-rate of children with foci of tuberculosis in the abdomen. Out of the 41 fatal bovine infected cases, 26 died in the first half of the year and 15 in the second, while in the case of the 10 children dying of human infections 8 died in the first half and 2 in the second.

(3) Cases in which the primary site of infection was considered to be in the cervical glands.

In 5 (2.3 per cent.) out of the 216 cases where tuberculous lesions were found at autopsy the cervical glands were the seat of the most extensive tuberculous lesions. The ages of these children varied from 5 months to 5 years. The distribution of the glandular lesions in these 5 cases was as follows:-

5 cases with tuberculous cervical adenitis.	1	Right sub-maxillary glands. Right upper cervical.
	1	The above glands on both sides of neck.
	1	Left sub-maxillary. Left upper and lower cervical.
	2	All the cervical glands (1 case right side. (1 case both sides. Upper mediastinal glands.

From this it will be seen that only in 2 cases was there spread of the lesion to the nodes in the upper mediastinum where the glandular lesions were not so extensive or as severe as in the cervical glands. In neither of these cases were the tracheo-bronchial glands involved.

In 2 of the cases there was evidence of an attempt on the part of the tissues to arrest the disease in the cervical glands. In one of these, that of a child aged

5/

5 years in whom most of the glands had been removed surgically some years before, one small fibrosed gland was found, while in another aged 4 years and 8 months some of the cervical glands showed early deposit of lime salts. Thus the age at which evidence of healing was noted in these two cases corresponded closely with that found in the case of thoracic lesions. Only in one case was any tuberculous lesion found in the tonsil, and in that, besides there being glandular spread to the upper mediastinal glands, there was a rather chronic tuberculous ulcer in the lower part of the ileum, with involvement of a few of the mesenteric glands. This abdominal lesion was considered related to the cervical one in view of the lesion in the tonsil. Unfortunately, no strain was isolated from the abdominal lesion, though from the cervical glands a human strain was obtained. In the 2 cases with extensive glandular lesions cerebral tuberculosis was present, while in one of these and another case there was generalised miliary tuberculosis. In the case which had been operated on and which showed the one fibrosed cervical gland, 3 very chronic tubercles about the size of hemp seeds were noted in the spleen. This observation was of interest as here the tubercle bacilli must have passed through the pulmonary circulation without causing any lesion, and, as was to be expected/

expected in view of the stage to which the healing process had advanced, we failed to isolate any organisms, either from the gland in the neck or in the splenic lesions. In 2 of the cases the tuberculous lesion had nothing to do with the cause of death, while in 3 the children had died with cerebral or generalised tuberculosis. There was failure to isolate the infecting virus in one case, as already described, while 2 bovine and 2 human strains were isolated from the remaining 4 children.

(4). Cases in which the primary site of infection was unknown.

(a) Of the 216 cases with tuberculous lesions 7 (3.2 per cent.) fell into this group. There were 4 cases in which, though complete autopsies were performed, no lesion was noted in the thoracic, cervical, or mesenteric glands. In 2 of these the only tuberculous lesion found was in the bones; in one of these in a child aged 7 months the lumbar vertebrae were involved, with the formation of a typical psoas abscess; in the other, a child aged 2 years and 4 months, the cervical vertebrae were the seat of a small tuberculous lesion, with the formation of a retro-pharyngeal abscess. In neither of/

of these cases was the tuberculous disease the cause of death, and the attempts to isolate the infecting virus were not successful. In the remaining 2 cases in this group the only tuberculous lesions found were in the brain. In one case there was a typical tuberculous meningitis,and in the other, in addition to this, a tuberculoma of the cerebellum was found. One child was aged 1 year and 9 months, and the other 3 years and 10 months, and from the lesions in the brain of both cases human strains were isolated. Both these cases were of interest as no macroscopic evidence of tuberculosis was found in any of the other organs or tissues. In both cases emulsions of mesenteric, bronchial and cervical glands were injected into guinea-pigs, with negative results. Histological sections of several glands from different groups in addition to the tonsils were also examined, but showed no evidence of tubercle. Even with these precautions it was possible that a small focus was missed, yet it may be that in each of these cases the inspired or ingested bacilli were immediately phagocytosed and passed into the blood stream, only to be carried to the vulnerable meninges. In the case of tuberculous lesions occurring in bones without a primary lesion being found elsewhere, Kaufmann (1929) was of the opinion that the bacilli gained entrance/

entrance to the body without producing a primary lesion at the portal of entry. Further, experimentally in the mouse, on which a large series of observations has been made, we often noted that the tubercle bacilli inoculated intraperitoneally were rapidly phagocytosed by small monocytes, polymorphs, and later by large macrophages, and within a few hours of the inoculation these tubercle laden cells were found in the pulmonary capillaries. Thus while phagocytosis may lead to the destruction of the tubercle bacillus, yet through the blood stream it must frequently cause the spread of the virus to more distant and more vulnerable parts.

(b) In 3 cases the post-mortem examination was limited at the request of the parents. In 2 of these only the head was examined and a typical tuberculous meningitis found. Isolation of the infecting virus was successful in only one of these cases, a human strain being obtained. In the remaining case the autopsy was limited to an examination of the cervical region where cario-necrosis of the fourth and fifth cervical vertebrae was noted, with an extra-dural abscess and compression of the spinal cord. No organisms were isolated from the material taken from the abscess.

The relation of the types of tubercle bacilli isolated from lesions found at autopsy to the results produced.

A. The distribution of the types of bacilli according to the age groups in which they were found and the cause of death.

(1) The tuberculous disease the cause of death.

Of the 165 fatal cases from which the infecting organisms were isolated (table XXII) the human type of virus was responsible for death in 120 (72.7 per cent.) and the bovine in 45 (27.3 per cent.). It is interesting to observe the fluctuations in the percentages of the two types throughout the various age-groups, the human type having been found in 80 per cent. of cases during the first 6 months of life and gradually falling during the second 6 months to its lowest level in the second year, viz., 62.2 per cent., after which it rose, with a slight fall in the 3 to 5 years' period, to 85.7 per cent. in the 5 to 12 years' group. Throughout similar age groups the percentage of the bovine infections was always much less than the human, rising gradually from 20 per cent. in the first 6 months of life to its highest point, 37.8 per cent. during the second year, i.e., at the time when the children were consuming large amounts of/

of cow's milk, and thereafter gradually falling to 14.3 per cent. in the 5 to 12 years' period, when it would appear that the chances of infection with the bovine type were less numerous, or what was more probable that the older child was more resistant to this type of infection.

The two youngest children dying of primary thoracic tuberculosis due to the human virus were both boys, the one aged 7 and the other 8 weeks, and have already been described in figs. 7 and 5 respectively. On the other hand, the youngest children with fatal abdominal tuberculous lesions from which the organisms were isolated were also two boys, each aged 17 weeks, the one being infected with the human type of bacillus and the other with the bovine. In the case with the human infection there was tuberculous peritonitis with widespread caseous lesions in the abdominal lymph nodes and scanty miliary foci in the various organs, but there was no evidence of meningitis and no obvious ulceration of bowel. In the bovine infected child tuberculous ulceration was present in the terminal part of the ileum, the mesenteric glands in relation being markedly caseous, and in addition there was a widespread generalised miliary tuberculosis and early tuberculous meningitis, but no peritonitis. The family histories of both were negative/

negative for tuberculosis. The bovine infected child came from a rural district, and had been fed with cow's milk from birth; the human infected case, on the other hand, was a city child, and had been fed on cow's milk only for the last 3 weeks of life, prior to which he had been breast-fed.

(2) <u>The tuberculous lesions not the cause of death</u>.

It was found that of the 18 tuberculous lesions which did not cause death, and from which the infecting organisms were isolated (table XXIII), 4 (22.2 per cent.) were infected with the human type and 14 (77.8 per cent.) with the bovine, though in 2 of the latter in which double portals of entry were present a fatal tuberculosis had resulted from thoracic lesions caused by the human type of virus, and it was from the mesenteric glands of one of these cases that the mixture of strains already described was isolated. The number of these non-fatal cases from which the infecting virus was isolated is small and does not allow of any very definite conclusions, though it is evident that in them the bovine type of infection was much commoner than the human.

B. <u>The association of the types of bacilli isolated with the lesions found at autopsy</u>.

For this study 123 human infections and 57 bovine were/

TABLE XXVII.

The lesions associated with primary thoracic tubercle due to human virus.

	0 to 2 yrs.	2+ to 5 yrs.	5+ to 12 yrs.	Total.
Pr. Thoracic human lesions	68	21	18	107
Generalised miliary tubercle	60 (88.2)	19 (90.5)	13 (72.2)	92 (86)
Cerebral tubercle (Meningitis	45 (66.2)	14 (66.7)	11 (61.1)	70 (65.4)) 76(71.0)
Cerebral tubercle (Tuberculomata.	(4m) .8. (11.8)	(2m) .3. (14.3)	(1m) .2. (11.1)	(7m) .13. (12.1)) 76(71.0)
Bone lesions	3 (4.4)	1 (4.8)	-	4 (3.7)
Tuberculides	4 (5.9)	1 (4.8)	-	5 (4.7)

(4m) in tuberculomata section indicates that in these cases meningitis was also present, and in the total cerebral lesions these double lesions have only been counted once.

Percentages in brackets.

were available, in all of which the infecting organisms were isolated from the primary lesion or lymph nodes associated therewith, and complete autopsies were made, while other 9 strains have not been included, these consisting of 6 strains isolated in duplicate (4 human and 2 bovine) from 6 cases with related abdominal and thoracic lesions, 2 bovine strains isolated from abdominal foci in which fatal human infected thoracic lesions were present, and one human strain obtained from a meningitis case in which only a limited post-mortem was allowed. In this section only general references have been made to the pathological lesions found as far as these have a bearing on the occurrence of the different strains, a more detailed study of such lesions having already been given.

(1) Human infections.

On considering the lesions associated with this type when the portal of entry was in the respiratory system (table XXVII), it is evident that an acute generalised miliary tuberculosis was commonest in the 2 to 5 years' period, occurring in 90.5 per cent. of the infections, while in the first 2 years it was slightly less common, being present in 88.2 per cent., and least common in the 5 to 12 years' group (72.2 per cent.).
Cerebral/

TABLE XXVIII.

Lesions associated with primary abdominal tubercle due to human virus.

	0 to 2 yrs.	2+ to 5 yrs.	5+ to 12 yrs.	Total.
Pr.abdominal lesions	8	2	2	12
Lung lesions other than miliary.	-	1 (50)	-	1 (8.3)
Generalised miliary tubercle.	6 (75)	1 (50)	1 (50)	8 (66.7)
Cerebral tuberculosis (Meningitis	5 (62.5)	1 (50)	1 (50)	7 (58.3))) 7 (58.3)
Cerebral tuberculosis (Tuberculomata	(m).1 (12.5)	(m). 1 (50)	-	(2m).2 (16.7))
Bone lesions	-	1 (50)	-	1 (8.3)
Tuberculides	-	-	-	-
Intestinal ulcers	2 (25)	-	-	2 (16.7)
Peritonitis	3 (37.5)	-	1 (50)	4 (33.3)

(m) = cases of tuberculomata associated with meningitis.
In the total cerebral lesions these double lesions have only been counted once, as in table XXVII.

Percentages in brackets.

Cerebral tuberculosis, under which is included meningitis and tuberculomata, was practically the same through all the age groups, though slightly commoner in the earlier years, while tuberculides and bone lesions, all of which involved the middle ear and mastoid region, were most frequently found in the first 2 years of life. It would appear from these results that as the child gets older there is less tendency for a lesion in the respiratory system caused by the human type of bacillus to generalise and cause other lesions.

The number of cases of primary human abdominal
1203.
infections is small (table XXVIII) and allows of no definite conclusion, though it would appear that generalisation of the infection was commonest in the first 2 years of life. Only one bone lesion - caries of the cervical spine - was noted, and in one case the lungs had become severely involved and were the seat of a sub-acute miliary lesion with areas of tuberculous broncho-pneumonia. On comparing the totals of tables XXVII and XXVIII it is evident that generalised miliary tuberculosis was commoner in the human infected thoracic lesions, occurring in 86 per cent. of cases as against 66.7 in the human infected abdominal lesion, a finding which was to be expected in view of the likelihood of invasion of blood channels in a vascular organ like the/

TABLE XXIX.

Lesions associated with primary abdominal tubercle due to bovine virus.

	0 to 2 yrs.	2+ to 5 yrs.	5+ to 12 yrs.	Total.
Pr. abdominal lesions	32	12	8	52
Lung lesions other than miliary.	2 (6.3)	-	1 (12.5)	3 (5.8)
Generalised miliary tubercle.	15 (46.9)	5 (41.7)	1 (12.5)	21 (40.4)
Cerebral tuberculosis (Meningitis	18 (56.3)	4 (33.3)	1 (12.5)	23 (44.2)) 24(46.2)
Cerebral tuberculosis (Tuberculomata.	(3m) 4 (12.5)	(m) 1 (8.3)	-	(4m) 5 (9.6))
Bone lesions	1 (3.1)	-	-	1 (1.9)
Tuberculides	4 (12.5)	-	-	4 (7.7)
Intestinal ulcers	6 (18.8)	3 (25)	-	9 (17.3)
Peritonitis	15 (46.9)	6 (50)	1 (12.5)	22 (42.3)

(m) = Cases of tuberculomata associated with meningitis. In the total cerebral lesions these double lesions have only been counted once, as in table XXVII.

Percentages in brackets.

the lung. Cerebral tuberculosis was also commoner in the thoracic lesion (71 per cent.) than in the abdominal (58.3 per cent.). The remaining 4 cases, from which human strains were isolated and which have not been tabulated above, consisted of 2 with primary foci in cervical glands and 2 cases of tuberculous meningitis in which no primary focus was found. The lesions associated with these 4 strains are, however, included in the totals in table XXX.

(2) Bovine infections.

Of the 3 bovine primary thoracic infections all were associated with generalised miliary tuberculosis and meningitis, and have not been tabulated separately, though included in the totals in table XXX. The lesions associated with the primary bovine abdominal infections have been tabulated in table XXIX. In 3 cases, though the disease was primarily abdominal, the lungs were severely involved, being the seat of sub-acute miliary tuberculosis with areas of tuberculous broncho-pneumonia in which there was early cavitation. Generalised miliary tuberculosis was found to be commonest in these cases in the first 2 years of life (46.9 per cent.), less common in the 2 to 5 years' period (41.7 per cent.), and slight in the 5 to 12 years' group (12.5 per cent.), while cerebral/

cerebral tuberculosis showed a similar predilection for the earlier years. Only one bone lesion, involving the mastoid region, was noted, while tuberculides, as in the case of the human thoracic lesions, were found only in the early years. Naked-eye evidence of intestinal ulceration was present in 17.3 per cent. of cases; in the remainder the bacilli had passed through the intestinal mucosa without causing any gross lesion, or the lesion had healed. Tuberculous peritonitis was almost four times as common from 0 to 5 as from 5 to 12 years. Other 2 bovine infections, with primary foci in the cervical region, have not been tabulated; in one of these generalised miliary tuberculosis, with tuberculous meningitis, was present, both these cases being included in the totals in table XXX.

(3) <u>Comparison of lesions associated with human and bovine infections</u>.

It is of interest to compare the lesions associated with the 12 primary human (table XXVIII) and 52 primary bovine abdominal infections (table XXIX). Severe lung lesions, generalised miliary tuberculosis and cerebral tubercle were all commoner in the case of the human infection, while the more localised tuberculous peritonitis was more often found to be associated with the bovine infection. Further, when the lesions associated with/

TABLE XXX.

Lesions associated with all human and bovine strains isolated from autopsy material.

	Human.	Bovine.
Total primary lesions - virus isolated - complete p.m.	123	57
Generalised miliary tubercle.	101 (82.1)	25 (43.9)
Cerebral tuberculosis (Meningitis	80 (65)) 86 (69.9)	27 (47.4)) 28 (49.1)
Cerebral tuberculosis (Tuberculomata	(10m) 16 (12.2))	(4m) 5 (8.8))
Bone lesions	5 (4.1)	1 (1.8)
Tuberculides	5 (4.1)	4 (7.0)

(m) = Cases of tuberculomata associated with meningitis.
In the total cerebral lesions these double lesions have only been counted once as in tables XXVII and XXIX.

Percentages in brackets.

with all the human and bovine infections were compared (table XXX), it was found that generalised miliary tuberculosis, cerebral tubercle and bone lesions were always commoner in the human infected cases; that is, the human type of infection is more virulent for the child as far as generalisation of the disease is concerned. This tendency to generalise on the part of such an infection was most marked in the human infected thoracic lesion where 86 per cent. of the cases had generalised miliary tuberculosis and 71 per cent. cerebral tubercle; while on the other hand the least tendency to spread of the infection was in the abdominal bovine infections where 40.4 per cent. showed generalised miliary lesions and 46.2 per cent. cerebral tuberculosis.

SECTION IV.

Surgical Tuberculosis.

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Surgical Tuberculosis.

Under this heading the types of tubercle bacilli associated with tuberculous lesions in the lymphatic glands, bones, joints and kidneys are considered. The data were derived from two sources:- (1) from 65 specimens sent from the surgical wards from which 52 strains (24 human and 28 bovine) were isolated; none of these cases proved fatal during their period of residence in hospital: (2) from 10 autopsy cases. In 4 of these the primary lesion was in the cervical glands (p.213), from which 2 human and 2 bovine strains were isolated, both human infected cases, and one bovine dying as a direct result of the tuberculous infection. In the remaining 6 cases the primary lesions (4 thoracic and 2 abdominal) were associated with tuberculous lesions in bones. In these cases the infecting virus was isolated only from the primary lesions or associated tuberculous lymphatic glands. Thus a total of 62 surgical lesions and associated strains are available for discussion.

A. Tuberculosis of lymphatic glands.

(1)/

TABLE XXXI.

Surgical Tuberculosis - 62 strains (31 Human and 31 Bovine) isolated from 65 surgical specimens and 10 autopsy cases.

Site of lesion.		0 to 2 yrs.	2+ to 5 years	5+ to 12 yrs.	Total.
Cervical glands		8	8	18	34
Type of infection	Human	2 (28.6)	3 (50)	5 (33.3)	10) 35.7% human
	Bovine	5 (71.4)	3 (50)	10 (66.7)	18) 64.3% bovine
	Lost or dead	1	2	3	6

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Cervical glands		8	8	18	34
Type of infection	Human	2 (28.6)	3 (50)	5 (33.3)	10) 35.7% human
	Bovine	5 (71.4)	3 (50)	10 (66.7)	18) 64.3% bovine
	Lost or dead	1	2	3	6
Other glands:- inguinal, axillary, mesenteric.		-	2	5	7
Type of infection	Human	-	-	2 (m.i.)	2) 33.3% human
	Bovine	-	1 (i.)	3 (m.i.a.)	4) 66.7% bovine
	Lost or dead	-	1 (a.)	-	-
Bones and Joints.		11	5	15	31
Type of infection	Human	8 (72.7)	3 (60)	6 (60)	17) 65.4% human
	Bovine	3 (27.3)	2 (40)	4 (40)	9) 34.6% bovine
	Lost or dead	-	-	5	5
Genito-urinary		-	-	3	3
Type of infection	Human	-	-	2	2) 100% human
	Bovine	-	-	-	-)
	Lost or dead	-	-	1	1

Figures in brackets are percentages.

a. = axillary glands.
i. = inguinal glands.
m. = mesenteric glands.

(1) Cervical glands.

In all, 34 cases of tuberculosis in these glands were investigated, and the infecting organism was successfully isolated from 28, 10 (35.7 per cent.) being of the human type and 18 (64.3 per cent.) bovine. In the remaining 6 cases isolation was not successful, either on account of the organisms being dead or due to technical difficulties. The age distribution of these cases is given in table XXXI. The bovine type of infection was slightly commoner in girls, occurring in 70 per cent. as against 61.1 per cent. boys, this feature being present in all the age groups. In 2 of the cases (S. 57, S. 18) the bovine strains isolated from the glands were less virulent than usual and these have already been discussed (table VIII).

The total bovine percentage of 64.3 found in this investigation is higher than that obtained in children in other countries. Griffith (1930 (2)) found on summarising the various investigations in English children under 15 years that 58.7 per cent. (44 out of 75) of cases of tuberculous cervical adenitis were infected by bacilli of the bovine type. Möllers (1928 (1)) in the collected statistics for this type of disease chiefly from German sources, and excluding those of Mitchell given below, noted that 40 per cent. of such infections/

infections in children under 5 years were bovine, while from 5 to 16 years the percentage was 26.2. On the other hand, the bovine percentage which we have obtained is lower than that given by other workers for Scottish children. Griffith (1915), in his investigation into cases of cervical gland tuberculosis from various parts of Scotland, found that in children up to 15 years, 10 (71.4 per cent.) out of 14 were infected with the bovine type. Mitchell (1914 (1 and 2)) found that of 80 cases of cervical gland tuberculosis in children under 13 years of age belonging to Edinburgh and the surrounding district 88 per cent. were bovine infections. The latter worker attributed this high percentage of bovine infections to the milk supply of the Edinburgh district. As already noted, over 90 per cent. of the milk supply in the Glasgow area is pasteurised, and this may account for the lower incidence of bovine tubercle found in this investigation. It is rather a striking fact in this connection that 16 (88.9 per cent.) out of the 18 children with bovine infections did not live in the Glasgow area, but came from country districts, and this greater percentage of bovine infections is more evident when it is remembered that the proportion of country to Glasgow children admitted during the period of this research was 13 : 23. Accordingly, it would appear from these results that in the case of tuberculous cervical adenitis, as/

as well as in the case of fatal abdominal tuberculosis due to bovine infections, the care, supervision, and pasteurisation of Glasgow's milk supply has probably some effect in reducing the number of bovine infections in Glasgow children.

(2) Glands, other than cervical.

From 7 cases of tuberculosis in lymphatic glands other than the cervical, 6 strains were isolated. Of these 2 were human and were obtained in the one case from the inguinal glands and in the other from mesenteric glands which were noted to be caseous at operation for appendicitis. The remaining 4 were bovine, 2 being obtained from inguinal glands, one from axillary, and one from mesenteric. The total incidence of bovine infections in these 6 cases was 66.7 per cent. In the cases with tuberculous inguinal and axillary glands no tuberculous lesion was noted clinically in the tissues draining into these lymph nodes. Such findings are not, however, sufficient evidence that a small lesion was not actually present in each case.

B. Tuberculosis of bones and joints.

In this section 20 strains were isolated from 25 specimens of surgical material. In other 6 cases which came/

came to post-mortem the bone lesion was found to be secondary to a lesion elsewhere, and the infecting viruses in these cases were isolated only from the primary lesions. In 4 of these cases the primary site of the tuberculous disease was in the lungs, the infection being of the human type and the associated bone lesions consisting of tuberculous disease of the middle ear and mastoid region. In another 2 cases with the primary lesion in the mesenteric glands there was caries of the cervical spine in one and a tuberculous mastoid in the other: in the former a human virus was isolated from the primary focus and in the latter a bovine.

The distribution of these 26 strains in the various bone and joint lesions is noted in the following table:-

Site of tuberculous disease.	Surgical specimens.		Autopsy specimens.	
	Human.	Bovine.	Human.	Bovine.
Mastoid	1	-	5	0
Humerus	1	-	-	-
Femur	3	-	-	-
Tibia	1	2	-	-
Fibula	1	-	-	-
Hip joint	1	4	-	-
Knee joint	1	1	-	-
Ankle joint	1	-	-	-
Cervical spine	1	1	-	1
Dorsal spine	1	-	-	-
Total	12	8	5	1

In 2 of the cases with bovine infections in the hip-joint occurring in boys aged 7 years the condition had been diagnosed radiologically and clinically as Perthes' disease. Of the 6 cases with the tuberculous disease in the mastoid 5 were found at autopsy as described above. This occasional involvement of the mastoid in cases where the primary focus was elsewhere is of interest as in 4 of the cases the lung lesions were severe and cavitation was present. We have noted that a very large number of cases of acute otitis media in infancy and childhood have been associated with acute broncho-pneumonia. The explanation of this would seem to be due to the fact that as the sick child lay on his or her back the highly infected secretions coughed up readily reached the openings of the eustachian tubes in the naso-pharynx, and consequently, as we have seen in many histological preparations, an acute inflammatory change was set up at the openings of these tubes, the lumen becoming plugged with desquamated cells and purulent exudate which rapidly spread back to the middle ears. The same sequence of events would appear to take place in some cases of severe pulmonary tuberculosis in children. In the remaining 2 children, both operated on for tuberculous mastoiditis, in one aged 11 months the infecting virus was human but no evidence of tuberculosis elsewhere was found clinically, while in the other case, aged 16 weeks, the child died as/

as a result of the operation, and at post-mortem caseous glands were found in the mesentery in the ileo-caecal angle, from which a bovine strain was isolated. In this last case the infection of the middle ear and mastoid was probably brought about through the blood stream as no other focus of tubercle was found anywhere in the body.

Of the bacilli associated with all the bone lesions in the present study 17 (65.4 per cent.) were human and 9 (34.6 per cent.) were bovine (table XXXI), so that the bovine percentage is much lower than that obtained by Fraser (1912) in Edinburgh children. This worker found that of 67 children under 12 years with bone and joint lesions 41 (61.2 per cent.) were bovine, 23 human, while in 3 lesions both types of bacilli were present. On the other hand, the bovine percentage we obtained is a little higher than the total obtained by Griffith (1930 (2)) in his various investigations into bone and joint tubercle in Scottish children, his percentage of bovine infection in children up to 15 years being 28.6 (8 out of 28), while in a very large series of English children under 15 years with bone and joint tubercle he found that only 20.9 per cent. (89 out of 426) were infected with the bovine type. Möllers (1928 (2)) in the collected data from 15 authors, excluding those of Fraser given above, noted that of 33 children under 5 years with bone/

bone and joint tuberculosis all were infected with the human type, while of 55 from 5 to 16 years only 5.45 per cent. were bovine infections.

Of the 17 human infections 9 were boys, and of the 9 bovine 5, so that both types of infection were found to be slightly commoner in male children, and this may be due to the fact that boys are more liable to knocks and bruises which having produced a small injury in a bone provide a focus for the tubercle bacillus to settle in and produce disease. The age distribution of these cases is given in table XXI.

C. Genito-urinary tuberculosis.

In 3 cases of tuberculosis of the kidneys, which were removed at operation, the human type of bacillus was isolated from 2; the isolation experiment failed in the third case. In all of these cases tubercle bacilli were being excreted in the urine, and clinically no primary focus of infection was discovered.

SUMMARY and DISCUSSION.

SUMMARY and DISCUSSION.

The investigation particularly concerns infants and children up to between 12 and 13 years of age admitted to the Royal Hospital for Sick Children, Glasgow, on account of illness or as the result of accident, over a period of six years - 1924 to 1930. They were, for the most part, children of poor or working-class people. In a series of 1,300 consecutive autopsies search was made for any evidence of tuberculous disease, the criteria for the diagnosis of which were the same throughout the whole series. Of the total 1,300 subjects 216 (16.6 per cent.) showed naked-eye evidence of tuberculous infection. From the surgical cliniques of the hospital, 65 cases of tuberculous lesions in bones, joints, lymphatic glands and kidneys were also studied, but none of these children died whilst in hospital.

For isolation of the infecting virus from specimens obtained from the above subjects the material was in nearly all cases treated with an ultimate concentration of 7.5 per cent. antiformin, washed twice with distilled water, and then cultured, although with some of the specimens where there was likely to be difficulty in isolation by culture guinea-pigs were inoculated subcutaneously as well. It was noted that even such a low concentration as/

as 7.5 per cent. of antiformin had an inhibitory effect on some strains, particularly of the bovine type. This was considered as being due to certain strains being more susceptible to the action of that agent, or to the fact that in some tissues the virus was not so well protected as in others. Accordingly, it is not possible to state in how many lesions the bacilli were dead when negative results were obtained on culture or after inoculation of guinea-pigs with antiformin sediment. When tissues were left in the ice-box for some days, the chances of isolating the infecting virus appeared to be less than if the experiments had been undertaken immediately after the specimen had been received. On the other hand, freezing at -5°C. for 1 to 56 days did not seem to have any disadvantage. In this connection it is possible, as shown by Richet (1927), that the fresh tissue may have had a lytic action on the tubercle bacillus, this being capable of acting at ice-box temperature which ranged between 5° and 18°C., but was not active at -5°C. Further observations on this point are, however, desirable.

For primary cultures of the organisms, egg media, prepared according to Wilson (1920), with and without the addition of 6 per cent. pure glycerin, were used. The amount and rapidity of growth on different batches of media varied to a moderate degree, even though everything in/

in the preparation was kept as standard as possible. This may have been due to uncontrollable differences in the eggs from which the media were prepared. Several tubes of egg media, with and without glycerin, were inoculated from each antiformin sediment or emulsion of fresh tissue. These were only plugged during the first 5 to 7 days of incubation with cotton-wool stoppers, after which they were hermetically sealed; this procedure was found to give better growth, probably on account of the free access of air, than when the tubes were sealed immediately after inoculation. Isolation of the infecting virus from the human material was successful by means of (a) antiformin and culture in 170 out of 263 experiments; (b) direct culture from fresh tissue in 12 out of 28 specimens; and (c) guinea-pig inoculation with 59 strains where (a) and (b) had failed. Of the 241 strains thus isolated 189 were obtained from tuberculous lesions found at autopsy, 6 of these being isolated both from primary and secondary lesions, making a total of 183 different strains. The remaining 52 strains were obtained from surgical material. The ages of the subjects from whom these organisms were obtained varied from 7 weeks to between 12 and 13 years. Human viruses were found to be easier to isolate in culture than bovine, particularly where the organisms were scanty in the material under investigation.

In/

In 3 cases culture on egg media gave positive results, while guinea-pigs inoculated subcutaneously at the same time and from the same specimens as used for culture, but with a dose many times larger, showed no tuberculous lesions when killed two months or more later. Further, no tubercle bacilli were recovered from these animals, either on culture or on inoculation of their tissues into a further series of guinea-pigs. On subcutaneous inoculation of 0.01 mg. of moist bacilli obtained from cultures of these 3 strains, lesions were produced in guinea-pigs, although both for this animal and the rabbit the virulence of these strains appeared to be lower than usual. From the cultural characters and the reactions produced after inoculation into guinea-pigs and rabbits these 3 viruses did not correspond to the avian type, but were like the human type of virus. Further, the lesions in the children from which they were isolated were not characteristic of those produced by avian bacilli. The irregular results in the first series of guinea-pigs were regarded as being probably due to the higher resistance of the animals used overcoming an infection with scanty bacilli of low virulence. A few other workers, already mentioned, have made similar observations to ours in this respect. Thus it would appear, in view of the existence of such strains, that culture/

culture of material suspected of containing the tubercle virus is worthy of trial, in addition to the inoculation of guinea-pigs, as by using both methods there is more security, with the possibility of an earlier diagnosis if the culture is positive.

For the determination of type of virus, the morphological characters were noted to be uncertain. The majority of strains (78.3 per cent.) ultimately typed as human were long and beaded, while almost 60 per cent. of those classified as bovine were short and showed no beading.

For a differential diagnosis of eugonic from dysgonic types, we made use of the cultural characteristics, and in particular the amount and rapidity of the growth in the primary culture and subsequent 4 sub-cultures where necessary, with the corresponding transfers to media containing glycerin (egg, serum and potato). The period of incubation of the cultures was from 4 to 6 weeks, with the exception of those put up for showing pigment. Of the 152 strains classified as human 134 were typically eugonic, and the majority of these produced pigment as well. Less eugonic growths were produced by the remaining 18 strains, ultimately typed as human, and in some of these the nature of the growth was almost similar to that given by the more freely growing bovine strains. Of the

89/

89 strains typed as bovine 77 were typically dysgonic in transfers made to glycerin media from the primary growth and subsequent 4 sub-cultures. Other 11 strains grew more freely on glycerin egg, but on glycerin potato the growth was particularly poor, and none produced pigment. The remaining strain, classified as bovine chiefly on account of the virulence test in rabbits, showed fairly marked eugonic characters on culture, but did not produce pigment.

After making due allowance for slight variation in the different batches of culture media, we found some strains of each type which approached the cultural characters of the other very closely, though with such it was nearly always possible to elicit one more or less specific characteristic, e.g., pigmentation, which helped towards a diagnosis of type. Accordingly, we are of the opinion that a sharp differentiation of the two types, while possible with most of the strains isolated, failed in the case of a few which had some cultural similarities. This is analogous with what is found in many other groups of bacteria, for example, the coliform, where the fermentative reactions of two bacilli quite different in their serological reactions may be absolutely comparable. While we found great difficulty in the classification of the more slowly growing human and the more luxuriant bovine/

bovine strains, yet possibly the former on further sub-culture beyond the stage to which they were taken in this investigation (the fourth sub-culture) would have shown more decided cultural characters. Griffith (1916 (2)), for example, found it necessary to carry on sub-culturing certain human strains as far as the twentieth generation before concluding that they were atypical. We noted in a few cases that by sub-culturing the less typical human strains beyond the fifth generation, the cultural characters became more satisfactory for purposes of classification; yet, on the other hand, many of the bovine strains treated in a similar manner, and having adapted themselves to a saphrophytic existence, produced growths which were much less readily classifiable than in early sub-cultures, even though no opportunity had been given for these strains to adapt themselves to media containing glycerin. Thus what had been gained on the one hand was lost on the other. We could, of course, have carried on the sub-culturing process with only doubtful human strains, but such a procedure would not have been uniform, as some importance was attached to making control observations by the comparison of the cultural results of the two types of the same generation.

An important point in the cultural field is the possibility of a diagnosis being made from the characters of/

of the growth as to the type of infection. Certainly, strains showing eugonic characters with yellowish or reddish pigmentation in primary cultures and early sub-cultures were found, from the results of the rabbit virulence test, to be human, and with this in mind, we did not proceed in the later stages of the investigation to type by animal experiment 54 strains derived from thoracic lesions in children dying as a result of the tuberculous disease. Further, cultures which remained dysgonic on the various differential media throughout all the 4 sub-cultures, and which did not produce pigment, were found to be bovine from the results obtained after the inoculation of rabbits. Apart from this, we feel it would be unwise to give a definite opinion as to type, though the results of culture were found to be of much value when correlated with those obtained by inoculation of animals.

Of the 241 strains isolated 181 (94 human and 87 bovine) were typed both by the results of culture and inoculation of rabbits. Of the 60 strains not tested for virulence 54, as noted above, were typically eugonic on culture, and it was not considered necessary to type these also by the animal test, while 6 (4 human and 2 bovine) had been isolated from secondary lesions and corresponded in their cultural characters to the strains obtained/

obtained from the primary lesions, which were typed both by culture and animal test. The intravenous method of inoculation was chiefly used for testing virulence in order to avoid any spread of infection to other animals in the limited space that was available for experiment from ulceration of the local lesion produced by subcutaneous inoculation. When the results of intravenous test were not satisfactory, it was repeated. On a few occasions the subcutaneous method of inoculation was used in the case of these repeat tests. The material for inoculation was taken from the primary, or one of the early sub-cultures,and cultures from 3 to 4 weeks' duration were used. The dose of the bacilli was estimated by weighing and then dilution. Any animals which were living at the end of 2 months were killed.

With 40 eugonic strains a dose of 0.1 mg. of moist culture was injected, but the lesions produced in 5 cases were severe, necessitating repetition of the test with a smaller dose. Using 0.01 mg. as the dose with 54 eugonic strains, minimal lesions resulted in 47, while moderate lesions were produced in 3, and severe in other 4 animals, one case of which was found to be due to a mixture of strains in the inoculated material, while another was probably due to a cross-infection contracted in the animal house. Of the various animals showing

severe/

severe reactions to human strains 4 were of a very purely bred type. With the 87 bovine strains a dose of 0.01 mg. was always used for inoculation of rabbits, and 77 of these died with generalised miliary tuberculosis in an average of 32 days, while in 8 death occurred from 45 to 60 days, and in 6 of these the cultures were less dysgonic than usual. The remaining 2 bovine strains isolated from cervical glands were less virulent than usual for the rabbit, and in both cases the cultures were less dysgonic than typical bovine strains.

On reviewing the evidence obtained from the reactions found in the inoculated rabbits, the irregular results experienced with some of the human strains can partly be explained as firstly due to the large dose (0.1 mg. intravenously) used for some of the experiments, and secondly perhaps to some of the animals used being more susceptible to infection than others, or to the particular strains under investigation being more virulent. There is little doubt that, as Oehlecker (1907) originally pointed out, the smaller the dose inoculated the greater the differences in the reactions produced by the two types of bacilli. Further, this worker was of the opinion that the difference in the lesions produced in animals was the important factor in the diagnosis of type and not the length of time the animals lived after inoculation/

inoculation. Even using the smaller dose (0.01 mg. intravenously) certain human strains found in this investigation caused fairly severe reactions. This may have been due to the lower resistance of individual animals, and, in particular, of the more highly bred types, e.g., the blue beveran, which were used in certain experiments. These were the only animals available at the time, and out of the 6 used in the whole research 4 showed extreme susceptibility to infection with human viruses, the inoculation test in all these cases requiring to be repeated before a definite diagnosis could be made. Fraenkel and Baumann (1906), in their extensive investigation already quoted, found that individual rabbits varied much in their sensitiveness to infection, and concluded that they were not suitable animals for testing virulence. If space had allowed us to use a series of animals for each virulence test in this investigation, we would have been able to eliminate reactions due to increased susceptibility to infection on the part of individual rabbits, and to have offered some opinion regarding differences in virulence, if any, of the strains producing irregular results on inoculation. In this connection the findings of Fibiger and Jensen (1908) are of importance as they concluded that there was a small number of strains which were intermediate between the human and bovine/

bovine types, either in respect of cultural characters or virulence. It is interesting to note that out of 12 such strains which they found 3 were obtained from lesions in children. While many workers would have classified strains showing variation in culture and virulence as atypical, we have preferred to regard such strains as variants of one or other of the types with which we were working. They have accordingly been classified as human or bovine when they showed one or more cultural features looked upon as characteristic of one of these types; the rabbit virulence test was, however, always regarded, in the case of these variant strains, as the chief test for differentiation.

In the study of the autopsy cases, the greatest number of cases with tuberculous lesions occurred in the first year of life and next in the second. Of the cases in which the primary site of infection was considered to be in the lungs or thoracic lymph glands, the greatest number (almost 40 per cent. of the total thoracic cases) was found in the children up to one year old. In the case of those in which the primary seat of the disease was considered abdominal, the largest number (almost 30 per cent. of the abdominal cases) was found in the second year of life. When the cases showing tuberculous lesions were expressed as a percentage of the total post-mortems, the/

the greatest percentage was found in the third year of life, and from the fourth year onwards the percentage was somewhat higher in girls than in boys.

Of the total 216 cases with tuberculous lesions 123 (56.9 per cent.) were considered as being due to infection primarily in the thoracic tissues (lungs or lymphatic glands). In connection with the pathway of infection of the lung and thoracic lymph nodes there has been great diversity of opinion. Villemin (1868) was probably the first to suggest that pulmonary tuberculosis was caused by inhalation of material coughed up by phthisical subjects. Koch (1886 and 1901), in view of his discovery of the tubercle bacillus, declared that infection took place almost solely by inhalation of dried pulverised sputum which contained the tubercle virus. In 1903, however, von Behring took a directly opposite view, and in his address at Cassell stated that infection occurred in infancy, and that the portal of entry of the tubercle bacillus was nearly always by the alimentary tract, and that the bacilli, having entered the body by this route, passed through the mucous membrane of the pharynx or intestine and the related lymphatic glands without producing any lesion. Such organisms, according to him, eventually settled in the lungs or bronchial glands, there to produce, either at once or after a period/

period of latency, typical tuberculosis when the resistance of the subject was lowered by disease or other cause. Calmette (1922) strongly supported this view, and quoted numerous animal experiments to prove it. The experimental data supporting these views have been discussed many times by different authors, and only a few of the experiments will be mentioned. For more extensive critical reviews of the subject, the reader is referred to McFadyean (1910) and to Cobbett (1917). In the majority of the experiments, animals were fed with relatively enormous numbers of tubercle bacilli, and many of the results so obtained are far from convincing as showing that the lungs could be readily infected without lesions first being produced in intestine or abdominal lymph nodes. In some of this work no precautions were taken to ascertain whether the older animals used were free of tuberculous infection before the start of the experiment, as, for example, in Calmette's and Guerin's feeding experiments with goats (1905), and the more recent experiments of Carvalho, Vidal and Santos (1929) in monkeys. In some of the experiments it is more than likely that with the huge doses of bacilli employed some may have been inhaled, and to overcome this possibility some experimenters introduced the bacilli into the stomach of the animal through a tube. Even here, however, the bacilli may have been aspirated into the trachea due to

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the tube setting up violent expulsive efforts on the part of the animal. Findlay (1914), in a carefully planned series of experiments where the bacilli were introduced into the stomach in a gelatin capsule, found that in rabbits in which pulmonary lesions were produced there was definite evidence of tuberculous disease in the bowel or mesenteric glands in all but one animal where there was some doubt about the intestinal lesion. Many of the workers conducting these feeding experiments seem to have lost sight of the fact that they were employing enormous doses of bacilli which swamped the normal resistance of the animal. Accordingly, rapid dissemination of the infection would be more likely to take place than if smaller doses had been used, and the humoral and cellular defences of the body allowed full play. Vansteenberghe and Grysez (1905), in an attempt to prove that it was possible for particulate bodies to pass from the lumen of the bowel to the lung, introduced carbon particles in the form of indian ink into the stomachs of guinea-pigs, and found that after a very short period the lungs and bronchial glands of old animals were diffusely infiltrated, while in the case of young animals this did not occur. In none of these experiments was any mention made of the findings in control animals, so that the carbon pigment may have been in the lungs before the start of the experiment. Cobbett (1910)/

(1910), working with B. prodigiosus and also tubercle bacilli, found that infection of the lungs and bronchial glands occurred much more readily by inhalation than by feeding. Other workers, including Kossel, Weber and Heuss (1905), Findel (1907), and Reichenbach (1909) used estimated doses of tubercle bacilli, and found that to infect an animal by the alimentary tract very much larger doses were required than when the animal was allowed to inhale the organisms. Of course, such experiments are open to the criticism that in the natural disease in the human subject, particularly the child, other modifying influences are at work, e.g., we have already mentioned the mild inflammatory infections of the respiratory system as lowering the resistance of the bronchial glands and laying them open to attack by the tubercle bacillus. Similarly, the mesenteric glands and bowel might be infected with much smaller numbers of tubercle bacilli if there was a mild enteritis present. Findlay (1914) has definitely shown this experimentally to be the case in rabbits. From this brief survey of the various animal experiments, it will be appreciated that the evidence obtained does not allow of any very definite conclusion regarding the alimentary system as the portal of entry of the infection in cases where tuberculous disease affects only, or is most advanced in, the lung.

With/

With this in view, we decided to look for the more or less characteristic primary lung lesion described by other workers as occurring in the human subject and corresponding to the primary site of infection. For a better understanding of the mechanism of infection and spread of the disease, we have discussed the anatomy of the lymphatic system of the lung as far as this has a bearing on the subject, and have pointed out that the lungs are fairly sharply marked off into definite lymphatic territories which drain into corresponding lymph nodes at the hilum. In our research into the existence of a primary lung lesion, we found such in 102 out of 123 cases in which the first site of infection was considered to be thoracic. Of the cases with primary foci 84 had one each, and in 18 they were multiple, though in 7 of these one of the foci was older than others. Thus in most of the cases the foci were single, and were most frequently the size of a hazel nut or smaller. All of them showed evidence of caseation, though in some further changes had taken place, e.g., cavitation, and a very few showed evidence of calcification or surrounding fibrosis on naked-eye examination. These primary lesions were rarely found in children under 3 months, and, with this exception, were most numerous in those under 3 years. Little evidence of healing was noted on naked-eye examination of such foci in the infant; only/

only 1 out of 50 showed evidence of fibrosis in children under 1 year, but with advancing age the tendency to heal became greater. Microscopically, however, evidence of fibrosis was found more frequently as the early stages of the reparative process could not be observed by the unaided eye. Calcification was also rarely observed on macroscopic examination, being found in only 6 out of the 117 primary foci. No completely fibrosed or calcified focus was found in the whole investigation, this finding being in marked contrast to that of continental and American workers. The reason for this difference in the occurrence of calcification in these primary foci and also of tuberculous lesions in the related tracheo-bronchial glands in children in different countries may be due to dietetic factors on which we are not competent to speak. We found these lesions most often in the right lung and in the upper third of the anterior aspect of the upper lobe on that side; the apex, however, was seldom the seat of a lesion. The tracheo-bronchial glands in direct anatomical relation to these foci were always diseased. The site of the lesion in any particular lobe was the important factor in determining the distribution of the glandular lesions. Foci in the upper lobes caused tuberculous adenitis in the superior tracheo-bronchial glands on the corresponding side; those in the lower lobes in the corresponding inferior/

inferior tracheo-bronchial, while those in the middle lobe on the right side produced tuberculous disease in the right inferior group. A few exceptions to this anatomical distribution of the tuberculous adenitis related to the foci were found, and these have been accounted for on anatomical grounds. Further, while the lymph nodes in direct anatomical relation were nearly always the seat of a tuberculous lesion, the disease had frequently spread to other groups of glands, and this was noted most often in relation to foci in the right upper lobe. As more foci were found in the right lung than in the left, the glands on the right side were more frequently the seat of tuberculous disease than those on the left. Both on naked-eye and microscopic examination the lesions in the primary focus and related lymph nodes were of the same age, or that in the glands more recent, so that a lymphogenous origin of the focus was unlikely. This was further supported by the fact that we never noted any retrograde spread from the tuberculous tracheo-bronchial glands back into the lung substance, and while in some of these there was spread of the tuberculous adenitis to the glands in the neck, or to the lymph nodes in the upper part of the abdomen, such was always secondary to that at the root of the lungs,as the intensity of the tuberculous adenitis diminished the further away it was traced from the glands in direct anatomical relation to the focus/

focus in the lung substance.

On studying the histology of these primary lesions, we noted that they were of the nature of a localised patch of tuberculous broncho-pneumonia, and thus due to inhalation of the tubercle virus. None of the primary foci could be demonstrated to have a vascular origin, and if such was found in the section, then these lesions were of secondary nature. Unlike secondary tuberculous broncho-pneumonic lesions found, for example, in cases with cavitation, many of these primary lesions showed at an early stage evidence of an attempt at healing. Out of the 67 foci examined microscopically 34 showed some surrounding fibrosis, and this was found to be commonest in children over 2 years. Microscopic deposits of calcium salts were found less frequently than fibrosis, but like the latter were found more often in children over 2 years of age than in those younger. The earliest stage at which we found these foci histologically consisted of a patch of tuberculous broncho-pneumonia which was undergoing caseous change and in which there were abundant tubercle bacilli. Cellular infiltration, early fibrosis and tubercle follicles were found at the edge. This fibrous encapsulation was more marked in the older foci, due partly to increase in the amount of fibrous tissue at the immediate edge of the focus, and also to the development of a secondary capsule from/

from the fibrous tissue formed around the secondary tubercle follicles in the neighbourhood. The latest stage at which we observed the primary lesion showed it to be well-encapsulated and sharply marked off from the surrounding tissue, and the seat of early calcification.

In the remaining 21 cases, which we regarded as having the primary seat of the infection in the thoracic tissues, no primary lung lesions were found, although in all of them some of the thoracic lymph nodes were the seat of a tuberculous adenitis. In 14 of these cases there were some points of similarity in the distribution of the glandular and pleural lesions to cases in which primary lung lesions had been found.

What was the significance of these primary foci? We have shown from the pathological and histological findings that a haematogenous or a lymphogenous origin was unlikely. If they were aerogenic, and this seems most probable, it is difficult to understand why single foci should be so much more frequently found than multiple, as a relatively susceptible subject like the young child would inhale large numbers of bacilli if in contact with a patient suffering from "open" pulmonary tuberculosis. Other unknown factors must accordingly be at work in determining the localisation of the lesion. It may, of course, be only the bacilli that reach the alveoli which are/

are capable of producing a lung lesion, those which are arrested in the bronchi either being destroyed by phagocytes, or passed to the lymph nodes for destruction, there to lie latent, or set up tuberculous adenitis. That the bacilli may be able to pass through the mucosa of the bronchi without causing any, or only minimal lesions is a possible explanation of those cases in this investigation in which, though the thoracic glands were the seat of tuberculous adenitis, no primary lung lesions were found, although the other possibility must be kept in mind that the lung focus was so small that it was missed. We regard both these possibilities as likely explanations of the thoracic cases where we found no primary lung focus. It seems to us that if the tubercle bacillus can be carried through the mucosa of the pharynx and intestine without producing lesions, or only minimal ones that heal rapidly, then the same must surely apply.to the mucosa of the trachea and larger bronchi. We are aware that many of the continental workers on the primary lung focus insist that this cannot occur, and that tuberculous tracheo-bronchial glands are always associated with a primary lesion in the lung. We are of the opinion, however, that this view is too extreme, and the possibility must be considered that infection of the tracheo-bronchial glands may occasionally occur from organisms passing through the mucosa/

mucosa of the bronchi. Similarly, the commonest site of the lesion in the upper part of the right upper lobe is equally difficult of explanation, and this also applies to the frequent localisation of pulmonary tuberculosis to the right apex in the adult. What other proof can we bring to help us in the solution of the problem, apart from those obtained from morbid anatomical and histological appearances, that these primary lesions are not due to haematogenous or lymphogenous origin? In the section dealing with the type of virus found in primary abdominal lesions, we have shown that 54 (81.8 per cent.) were bovine and 12 (18.2 per cent.) human, while in the cases which we considered as primary thoracic infections 107 (97.3 per cent.) were human and 3 (2.7 per cent.) bovine. Further, from cases in which the primary lung lesion was found, only human types of bacilli were isolated, either from the lesion itself or from the glands in direct anatomical relation. If the focus which has been described as the primary lesion were of haematogenous origin, we should have found bovine bacilli in view of the fact that 81.8 per cent. of the primary abdominal lesions were due to the bovine virus. For example, in the case of the tuberculous lesions in bones and joints in the study on surgical cases, we found that 9 out of 26 (34.6 per cent.) of the lesions were infected with the bovine type of virus.

Tuberculous/

Tuberculous lesions in these structures are universally regarded as having a haematogenous origin. Also in favour of the aerogenic origin of these cases is the fact that 91 out of 105 (86.7 per cent.) children with human infections were city children who, in consequence of the overcrowding associated with towns, run a greater risk of air-borne infections. Thus from the pathological, histological and bacteriological points of view, the primary lung lesion would appear to be undoubtedly of aerogenic origin.

In the 3 thoracic cases in which bovine bacilli were isolated, no primary lung lesions were found. The portal of entry, as already suggested, in these cases may have been other than thoracic, viz., pharyngeal or abdominal, and if so, the infecting virus must have passed through the sub-maxillary and cervical glands on the one hand, and through the lower mediastinal on the other, without causing any lesion. Such a view would be in keeping with the teachings of von Behring and Calmette, yet from the morbid anatomical appearances in 2 of the cases the glandular lesions in the tracheo-bronchial glands corresponded closely to cases in which human types of bacilli were isolated, and in which primary lung foci were found. Thus it would seem likely that the infection in these 2 cases may have come from the lung. In the case with/

with the most advanced lesion in the para-tracheal glands from which a bovine strain was isolated the pathological evidence was not so convincing. If we regard these 3 cases as due to inhalation of the virus, the question arises - from where and how did these children contract so rare a type of primary respiratory infection? That there are "open" respiratory carriers of the bovine type of virus is known, and the literature on this subject summarised by Griffith (1930 (1)) shows that they are commoner in Scotland where they constitute 4.5 per cent. of patients expectorating viable tubercle bacilli as against 1 per cent. in England, and 0.3 per cent. on the continent. Accordingly, one would expect to find the readily infected respiratory system of the child more often the seat of a primary tuberculous infection due to the bovine virus in Scotland than elsewhere. This suggested the possibility that these 3 cases reported here might have been related to the same focus of infection, but on investigation it was found that they lived in widely separated parts of the Glasgow area, and, further, they were admitted to the hospital at intervals of 6 to 21 months from each other.

Accordingly, to us it seems most likely that in the young child at least the commonest way by which the respiratory system is primarily infected by the tubercle

virus/

virus is through the air passages. We do not deny - and, as a matter of fact, have shown - that in some cases of primary abdominal tuberculosis severe secondary pulmonary disease may be met with.

On considering the question of the relation of latent tuberculous infection in the tracheo-bronchial glands to these primary lung lesions, certain factors must be taken into consideration. As we have already observed, several workers have found tubercle bacilli in apparently normal tracheo-bronchial glands. The question arises - did there exist in such cases primary lung lesions which were missed at post-mortem? Such is a possibility, as the infection which these workers found may have been contracted shortly before the child died. None of the workers on latency have been able to give a definite opinion on the duration of such, though it has been suggested by some of them that it may have been in existence for a few weeks, while others hold that the bacilli may lie latent for several years; but in expressing such opinions they give no convincing clinical or pathological data with which to support them. This question of latency and lymphatic permeation of the bacilli without causing definite lesions is deserving of much further investigation in a specially selected series of cases in children where the chances of recent infection, for example, with the bovine virus,/

virus, could be excluded.

In the majority of the cases regarded as primary thoracic infections, the lungs were the seat of secondary miliary or broncho-pneumonic lesions, the latter being due in most cases to the breaking down of the primary lung lesions. Pulmonary tuberculosis as we saw it in the infant and child was, for the most part, an acute disease, only 5 out of the 123 cases being of chronic nature, and all of these children were over 4 years and 9 months. Rupture of enlarged caseating bronchial glands into the bronchi was found to be rare, and so it was unlikely that the primary lung foci were due to such a lesion. Haemorrhage from erosion of large vessels was found to be also uncommon in the child as compared with the adult. When pleural changes were present, these were found in the great majority of cases on the same side as the primary lesion and were due to spread of the chronic inflammatory process from the superficial lung lesion to the pleural tissues. When there was any breaking down of the lung focus, multiple acute tuberculous ulcers were found in the bowel. In a proportion of the cases the disease had spread to the cervical and abdominal glands, and it was noted that the cervical glands were most often involved by direct spread from below upwards (lower deep cervical glands), less often indirectly by the sputum causing lesions/

lesions in the pharynx or larynx. On the other hand, the abdominal glands were most frequently involved indirectly by infection through the sputum. In only 3 children, all of whom were over 2 years of age, out of the total 123 cases which we considered primary thoracic infections had the tuberculous disease not been the cause of death. In these cases the tuberculous disease was of limited extent, and had been unsuspected during life.

In 84 cases (28.9 per cent.) of the total 216 cases with tuberculous lesions, the pathological evidence was in favour of the site of the first infection being in the abdomen. Of these, 15 showed naked-eye evidence of intestinal ulceration, chiefly in the lower ileum, and all the subjects who were under 5 years of age died of tuberculosis. All the children under 1 year of age with intestinal ulcers were fed on cow's milk. In the remaining 69 cases no ulcers were noted, but the mesenteric glands were the seat of tuberculous adenitis. In 11 of these, the majority of which were due to infections with the bovine virus, there was evidence of healing of the lesion in the lymphatic glands. In the cases with intestinal ulceration, there was more extensive involvement of lymphatic glands, and secondary lesions were more frequent than in those without ulcers, and, further, all the cases with ulcers were fatal, while of the 69 cases without ulcers/

ulcers only 71 per cent. died of tuberculous disease. In cases showing evidence of healing in the mesenteric glands there were few secondary lesions, and the majority (8 out of 11) were found accidentally post-mortem. There were fewer secondary lesions and less extensive glandular involvement in the abdominal cases as compared with thoracic. Abdominal tuberculous lesions were found to be less fatal than thoracic, 20 out of 84 (23.8 per cent.) not causing death, and such non-fatal lesions in the abdomen were found in younger children than in the thorax.

From these abdominal lesions, 12 human and 54 bovine strains were isolated. Country children were slightly more often the victims of bovine infection than city children, possibly on account of most of the milk supply to the Glasgow area being pasteurised before distribution. In breast-fed babies the incidence of both types of infection, but particularly the bovine, was very much lower than in the corresponding bottle-fed children. The greatest number of infections with the human type of virus in the abdominal cases was found in children aged 9 to 18 months, i.e., at the period when children commence to move about themselves, and consequently are more liable to pick up infection from the ground and floors. The high incidence of the bovine type of infection in the abdominal cases is doubtless due to the ingestion of infected/

infected milk, as there was a greater incidence of this type of infection in bottle-fed babies and also for the 6 months to 2 year age groups, when the children were consuming large amounts of milk. But the reason for this greater frequency of the bovine type of infection in children in Scotland as compared with other countries is inexplicable, as we can find no reliable veterinary statistics which would be comparable regarding the incidence of "open" tuberculosis in cattle. Whether climatic or dietetic factors have a bearing on the subject is difficult to say. In Scotland, as compared with the continent, there is less sunlight, particularly in the winter months, and also the diet of the poorer class child, with whom the investigation was particularly concerned, may be different from that of the continental child, probably less foods (vegetables and fats) rich in vitamins A and D being consumed. It may be that a combination of these factors renders the Scots child less resistant than the continental to infection with the less pathogenic bovine virus. That pasteurisation of the milk supply of the city area seems to be effective in reducing the numbers of this type of infection is seen from the fact that country children, both in the case of abdominal and cervical gland tuberculosis, were more frequently infected with the bovine virus than city/

city children, though admittedly the numbers available for comparison are small. The fact that a greater number of non-fatal bovine infections was found in children over 2 years than under this age suggests that the older child is more resistant to such infection as compared with the human type of virus which, in the case of tuberculous abdominal lesions, was found to be frequently associated with a positive family history as was also noted in the human infected thoracic cases.

In 12 cases the portal of entry appeared to be elsewhere than in the thorax or abdomen. In 5 the oldest lesions were in the cervical glands, and in 2 of these the glandular lesions had spread into the nodes in the upper part of mediastinum, but had not involved the tracheo-bronchial glands. From these, 2 human and 2 bovine strains were isolated, while from the remaining case, on account of the advanced stage of healing, no strains were obtained. In 2 cases tuberculous disease was found in bone and in other 2 in the meninges, but in none of these was a primary source of the infection discovered. In the remaining 3 cases the autopsies were limited to the diseased areas. From all these 12 cases, with the primary lesion elsewhere than in thorax or abdomen, 5 human strains and 2 bovine were obtained.

Of 165 fatal cases from which the infecting bacilli were/

were isolated, 120 (72.7 per cent.) were of the human type, the greatest percentage of these as compared with bovine occurring in the first 6 months of life and from 5 to 12 years, while 45 (27.3 per cent.) were of the bovine type, and the proportion of these to the human type throughout all the age groups was always lower, the highest percentage (37.8) being in the second year of life, i.e., at the period when the child is consuming large amounts of raw milk. The youngest cases dead of primary thoracic tubercle, and from which the infecting bacilli were isolated, were aged 7 and 8 weeks respectively, both being infected with the human type of bacillus, while in the primary abdominal group the two youngest were both aged 17 weeks, one being infected with a human strain and the other with a bovine. From 18 autopsies where tubercle was not the cause of death, and in which the infecting virus was isolated, 4 were of human, and 14 (77.8 per cent.) of bovine type. This is suggestive that the bovine type of infection is less fatal than the human. As already noted in the case of the mesenteric glands, those infected with the bovine type showed a greater tendency to heal than those infected with the human. Of the total 183 different strains isolated from autopsy material, 59 (32.2 per cent.) were bovine, the percentage of bovine infections being very much higher than that recorded for autopsy material

from/

from continental children, and just less than twice as high as that for similar material from English children. In children under 2 years the bovine percentage was 30.9 (35 out of 113), from 2 to 5 years 36.6 (15 out of 41), and from 5 to under 13 years it was 31 (9 out of 29), table XXXIII. As already noted, no attempt was made to investigate the incidence of latent tuberculosis in the various groups of lymphatic glands which appeared normal to the naked-eye. The above results thus represent the frequency of the human and bovine types in actual tuberculous lesions, and are not strictly comparable with those of other workers quoted below, as in their investigations the question of latency was also taken up. Bearing this in mind, however, it is possible to compare the frequency of the two types of tubercle bacilli found in different countries in children's tissues. In the Local Government Board's (1914) investigation in 195 London children aged from 2 to 10 years, the infecting virus was successfully isolated in 98 autopsy cases, in one case both types of bacilli being present, and of these 99 strains 18 (18.2 per cent.) were of the bovine type, while in our series the bovine percentage in the corresponding age group is 34.3. Gaffky (1907) investigated 300 autopsies on Berlin children from 0 to 13¼ years for the presence of the tubercle virus, while Rothe (1911) continued Gaffky's work/

TABLE XXXII.

Comparison of strains isolated from autopsy material from children.

Country	Author	No. of Autopsies	Strains isolated. Total	Human	Bovine	Atypical	Bovine Percentage.
England.	Eastwood, A.S. & F. Griffith.	195	99*	81	18	-	18.2
Germany	Gaffky	300	57	55 [a.]	2 [b.]	-	3.5
"	Rothe	100	21	20	1	-	4.8
"	Ungermann	171	39	37	2	-	5.1
"	Total	571	117	112	5	-	4.3
Norway	de Besche	134	51*	46	4	1	7.8
Scotland	Blacklock	1,300	183	124	59	-	32.2

* In one case mixed strains isolated and counted as 2 strains in above table.

a. Gaffky gives this figure as 53, as 2 strains were still under investigation. Rothe, who continued Gaffky's work, gives the figures as above.

b. In these 2 cases Gaffky was unable to cultivate the bacilli from guinea-pig lesions in spite of repeated attempts over 1½ years. He concluded partly on these grounds that they were bovine.

work, but only in children up to 5 years. Ungermann's (1912) investigation, also in Berlin, included children from 1 month to 10 years, while de Besche (1913), in his investigation in Christiania, examined the lymph glands of children aged from over 1 month to 15 years. The results of these various investigations in addition to our own are given in table XXXII, from which it is evident that the bovine percentage found in autopsy material from Scottish children is much higher than in English or continental. This is partly due to the greater incidence of primary abdominal tuberculosis in Scottish children due to the bovine virus.

On investigating the various lesions with which the different strains were associated, it was found that in primary thoracic tuberculosis due to human infections, generalised miliary tuberculosis, and bone lesions were most commonly found in the first 5 years of life, while cerebral tuberculosis had much the same incidence throughout all ages. Generalisation and cerebral tuberculosis were more commonly associated with human infected thoracic lesions than with similarly infected abdominal lesions. All 3 primary thoracic lesions due to the bovine virus were associated with generalised miliary tuberculosis and meningitis. In the primary abdominal lesions due to bovine virus, cerebral tuberculosis and tuberculous

peritonitis/

peritonitis were most often present in the first 2 years of life and least from 5 to 12 years. Generalised miliary tubercle and cerebral tuberculosis were more commonly associated with abdominal tuberculosis due to the human type than with that due to the bovine, though with the latter, tuberculous peritonitis was more common. On comparing all the human and bovine strains isolated and the lesions found at autopsy, generalised miliary tuberculosis, cerebral tuberculosis, and bone lesions were most commonly associated with infections of the human virus, so that generalisation was found to be more frequent from an infection with the human type than a bovine. How far this depends on the type of infecting virus and situation of the primary lesion is difficult to say. As we have already observed, generalisation of the disease from lesions in the abdomen was less frequent than from lesions in the thorax. Probably both factors, viz., type and site of infection, play a part.

From surgical material 52 strains were isolated, while in 10 autopsy cases tuberculous disease was present as the primary lesion in cervical glands in 4, and in 6 subjects with the primary focus elsewhere there was a secondary tuberculous lesion in bones, the virus in these cases being isolated only from the primary lesion. Of 28 cases of tuberculous cervical adenitis 10 were infected with/

with the human type and 18 (64.3 per cent.) with the bovine. This percentage is lower than that found in other investigations in Scots children, though higher than that found in English and continental children. The bovine type of infection in these glands was commoner in country than in town children. In 6 cases where glands other than cervical were the seat of tuberculous disease, 2 were human infections and 4 were bovine. In 26 cases of tuberculosis of bones and joints, 17 were due to infections with the human virus and 9 (34.6 per cent.) with the bovine, the incidence of the latter being practically the same in town and country children. The incidence of the bovine infection in bone and joint tubercle is slightly higher than that recorded for English children, and much higher than that given for continental children. From 2 cases of genito-urinary tuberculosis human bacilli were isolated.

In table XXXIII the proportions of the two types of bacilli found in this investigation and their source are summarised.

TABLE XXXIII.

Summary of all strains isolated. ✱

Type of Case.	Total Strains	0 to 2 years			2+ to 5 years			5+ to 12+ years.			Total Bovine Percentage
		H.	B.	B%	H.	B.	B%	H.	B.	B%	
Autopsy Cases. (Dead of Tuberculosis	165	78	31	28.4	24	11	31.4	18	3	14.3	27.3
Autopsy Cases. (Dead of other causes	18	-	4	100.	2	4	66.7	2	6	75.	77.8
Surgical cases	52	6	6	50	3	5	62.5	15	17	53.1	53.8

✱ 6 strains (4 human and 2 bovine) isolated from abdominal as well as from thoracic lesions in cases in which such lesions were related have only been included once in the above table.

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A STUDY OF THE BACTERIOLOGY AND PATHOLOGY OF TUBERCULOUS DISEASE IN INFANCY AND CHILDHOOD

(Figures)

by

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Fig. 1. Diagramatic representation of a primary lobule, with its blood-vessels and lymphatics.

Br. = a bronchiolus respiratorius which divides into two ductuli alveolares = d.al., only one of which is carried out in detail; a.a.a. = three atria each of which communicates with a number of sacculi alveolares = s.al., around the periphery of which and opening into it are situated the alveoli = c. Alveoli are also connected with the bronchiolus respiratorius, ductuli alveolares and atria. P.= pleura.

P.A.= pulmonary artery which divides into three atrial branches, and these divide into branches which are distributed to the sacculi alveolares; from these latter branches the capillary network in the walls of the air spaces arises.

P.V.= pulmonary vein which is made up of branches which arise (I), from the pleura, (II), from the distal end of the ductuli alveolares, (III), from the walls of the sacculi alveolares, (IV), from the neighbourhood of the place where bronchi or bronchioli divide. The lymphatic plexus in the walls of the bronchioles divide into 3 branches at the distal end of the ductuli alveolares, (1) joins the lymphatic plexus around the pulmonary artery, (2) and (3) join the plexus around the pulmonary vein. The position of the lymphoid tissue is indicated by the stippled areas. After W.S. Miller, Amer. Rev. of Tuberc.

Fig. 1.

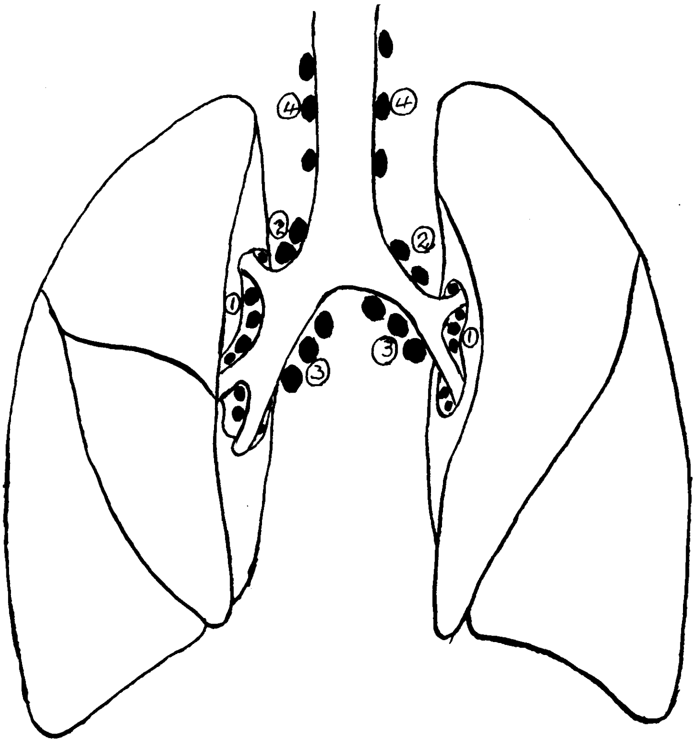


Fig. 2.

Diagram showing the extra-pulmonary group of tracheo-bronchial lymph nodes.

(1) Extra-parenchymatous broncho-pulmonary glands.
(2) Superior tracheo-bronchial glands.
(3) Inferior tracheo-bronchial glands.
(4) Thoracic paratracheal glands.

Fig. 3.

Key to diagrams. Description of the various symbols used in recording the type of lesions found in lungs, pleura and tracheo-bronchial glands.

Lung.

= Acute or sub-acute miliary tubercles.

= Caseous focus.

= Caseous focus with surrounding tubercles.

= Caseous focus with fibrosis around.

= Caseous focus with fibrous puckering of pleura.

3 3 3 3 3 = Tuberculous broncho-pneumonia.

= Acute cavity.

= Cavity with caseous walls.

= Cavity with caseous walls with fibrosis around.

= Pleural adhesions.

Lymph Glands.

= Completely caseous gland.

= Gland with caseous foci.

= Normal gland.

= Partly caseous gland.

= Spread of glandular lesion upwards.

= Spread of glandular lesion downwards.

Fig. 4. Three chronic foci, probably of different ages.

Case 2596. Male, aged 11 years. No family history of tubercle.

Post-mortem. Lungs - See diagram. Three foci were of chronic nature. Some chronic peri-bronchial lymphatic tubercle, and patches of acute tuberculous broncho-pneumonia in posterior parts of left upper lobe and both lower lobes.

Thoracic glands - See diagram. Most involved on right side.

Pleura - Right-sided chronic adhesive pleurisy.

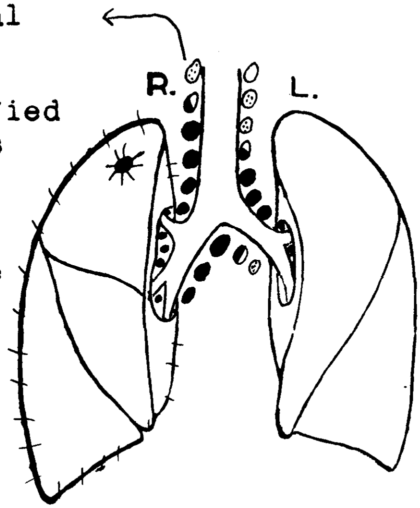
A few miliary tubercles in spleen; extensive tuberculous ulceration of small and large bowel; caseous mesenteric glands; no meningitis.

Histology. Focus in upper lobe showed advanced calcification, with dense fibrosis around. Those in left upper lobe and right lower were the seat of cavitation, and showed no evidence of calcification. The fibrosis around was not so dense as that in the right upper lobe.

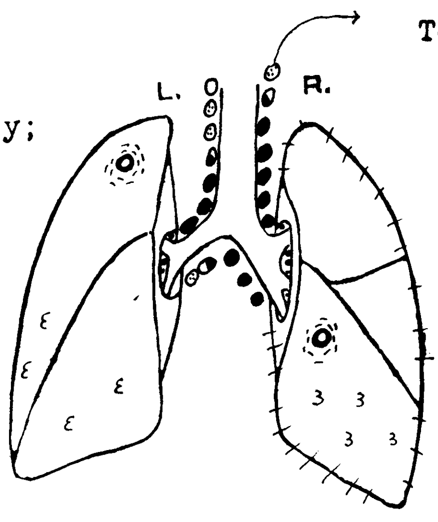
Bacteriology. Human strain isolated from right tracheo-bronchial lymph nodes.

Discussion. In this case the chronic pleurisy and more extensive glandular involvement on the right side suggested that the primary focus was on this side. On microscopic examination the focus in the right upper lobe showed more advanced histological change (fibrosis and calcification) than the others, and was considered primary.

Fig. 4.



ANTERIOR.



POSTERIOR.

Fig. 5.

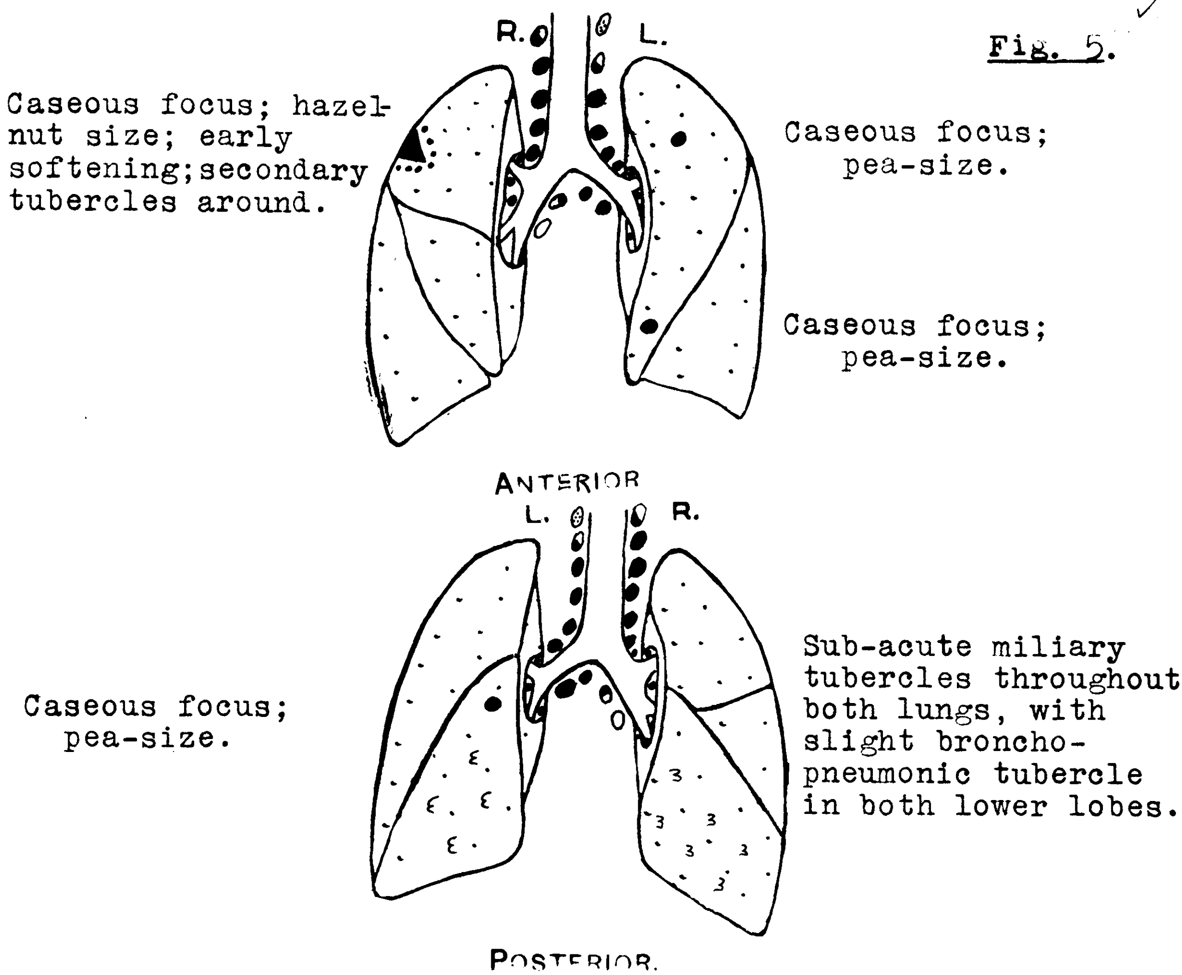


Fig. 5. Case with multiple acute foci, one being probably primary, in right upper lobe.

Case 2558. Male, aged 2 months. No family history of tubercle.

Post-mortem. Lungs - See diagram.

Thoracic glands - See diagram.
Slight generalised miliary tuberculosis; no meningitis.

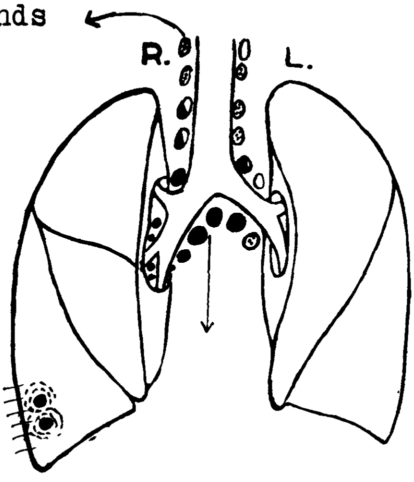
Histology. The focus in the right upper lobe showed numerous secondary tubercles around, in addition to some early fibrosis at places. There was early softening of the caseous material. The other 3 foci showed no secondary tubercles and no fibrosis, and were areas of tuberculous broncho-pneumonia, with caseous change, or were due to vascular lesions (see figs. 29a and 29b).

Bacteriology. Human strain isolated from right tracheo-bronchial glands.

Discussion. The focus in the right upper lobe was considered primary on account of the secondary tubercles around, and also the early fibrosis.

To lower cervical glands

Fig. 6.



2 pea-sized foci; caseous; fibrosis around; chronic pleurisy.

ANTERIOR.

Fig. 6. Two chronic foci of same age.

Case 1946. Female, aged 9 years. No family history of tubercle.

Post-mortem. Lungs - See diagram.

Thoracic glands - See diagram. Most extensive involvement on right side. Broncho-pulmonary to right lower lobe markedly caseous; same glands on the left side not affected.

Slight spread of the tuberculous lesion traced through lymphatic glands to right lower triangle of neck, and down through diaphragm to nodes in neighbourhood of coeliac axis.

Death due to operation following removal of appendix.

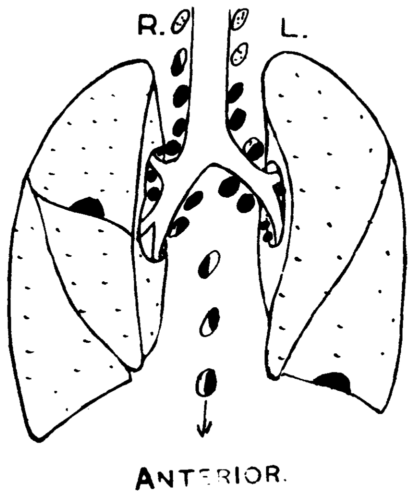
Histology. Both nodes showed much fibrous encapsulation, but no calcification. The pleura over the foci was the seat of chronic fibrosis. (See figs. 46a and 46b).

Bacteriology. Human strain isolated from right inferior tracheo-bronchial glands.

Discussion. In this case it was obviously not possible to distinguish between the age of the two foci, and both were considered primary.

Fig. 7.

Caseous pea-sized focus.



Both lungs sub-acute miliary tuberculosis; slight broncho-pneumonic tubercle.

Caseous pea-sized focus.

<u>Fig. 7.</u> <u>Earliest age at which primary lung foci were found.</u>

<u>Case 2866.</u> Male, aged 7 weeks. No family history of tubercle.

<u>Post-mortem.</u> <u>Lungs</u> - See diagram.

<u>Thoracic glands</u> - See diagram. Direct spread of lymphatic gland lesion through diaphragm to lymph nodes in upper part of abdomen.

Marked generalised sub-acute miliary tubercle; no meningitis.

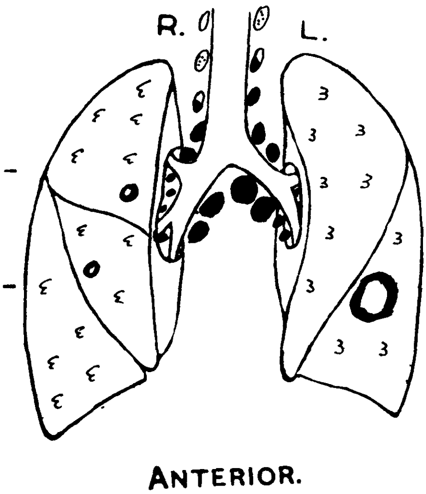
<u>Histology.</u> Both foci caseous; that in left lower lobe showed early breaking down. No fibrosis. Slight cellular reaction around - chiefly lymphocytic, with a few endotheliod cells.

<u>Bacteriology.</u> Human strain isolated from inferior tracheo-bronchial glands.

Fig. 8.

Cavity - hazel-
nut size;
caseous walls.

Cavity - hazel-
nut size;
caseous walls.



Tuberculous
broncho-pneumonia
both lungs.

Cavity - caseous
walls; walnut-size.

Fig. 8. Earliest age at which cavities were found.

Case 2381. Male, aged 4 months ($17\frac{1}{2}$ weeks).
Mother dead of phthisis 5 weeks before.

Post-mortem. Lungs - See diagram.

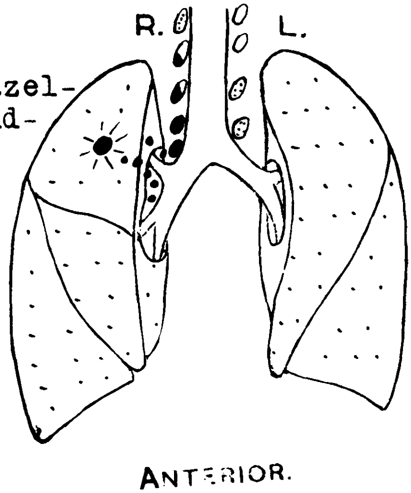
Thoracic glands - See diagram. Moderate generalised miliary tuberculosis; no meningitis. Extensive tuberculous ulceration of small and large bowel; caseous mesenteric glands.

Histology. No evidence of fibrosis or calcification in any of the cavities.

Bacteriology. Human strain isolated from left tracheo-bronchial glands.

Fig. 9.

Caseous focus; hazel-
nut size; surround-
ing fibrosis;
puckering of
pleura.



Sub-acute miliary
tubercles through-
out both lungs.

Fig. 9. Earliest age at which evidence of healing noted macroscopically.

Case 2454. Female, aged 7½ months. No family history of tubercle.

Post-mortem. Lungs - See diagram.

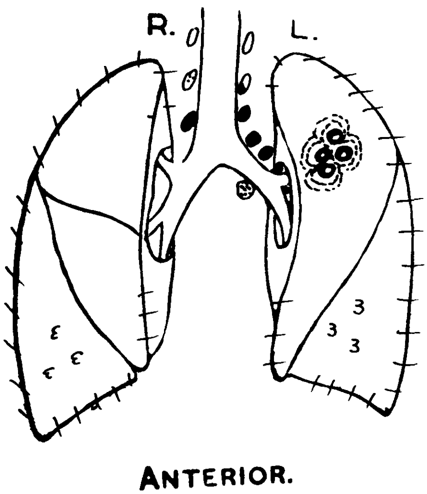
Thoracic lymph nodes - See diagram. Those on right side most involved, particularly to right upper lobe.

Slight generalised miliary tubercle (spleen and lungs); tuberculous meningitis.

Histology. None.

Bacteriology. Human strain isolated from primary lesion and right superior tracheo-bronchial lymph nodes.

A few patches of tuberculous broncho-pneumonia in both lower lobes.



4 pea-sized, caseous-walled cavities, with fibrosis around.

Marked chronic adhesive pleurisy on both sides.

Fig. 10. Youngest case to show an attempt at healing of cavities.

Case 2442. Male, aged 1 year 11 months. No family history of tubercle.

Post-mortem. Lungs - See diagram.

Thoracic glands - See diagram. Left superior tracheo-bronchial most involved; only isolated foci in left inferior tracheo-bronchial. Chronic pericarditis; chronic peritonitis in relation to diaphragm. No generalised miliary tuberculosis. Tuberculous meningitis. Extensive tuberculous ulceration of bowel (sputum infection), with caseous mesenteric glands.

Histology. Well-marked fibrosis around all cavities in upper lobe, but no calcification.

Bacteriology. Human strain isolated from left superior tracheo-bronchial glands.

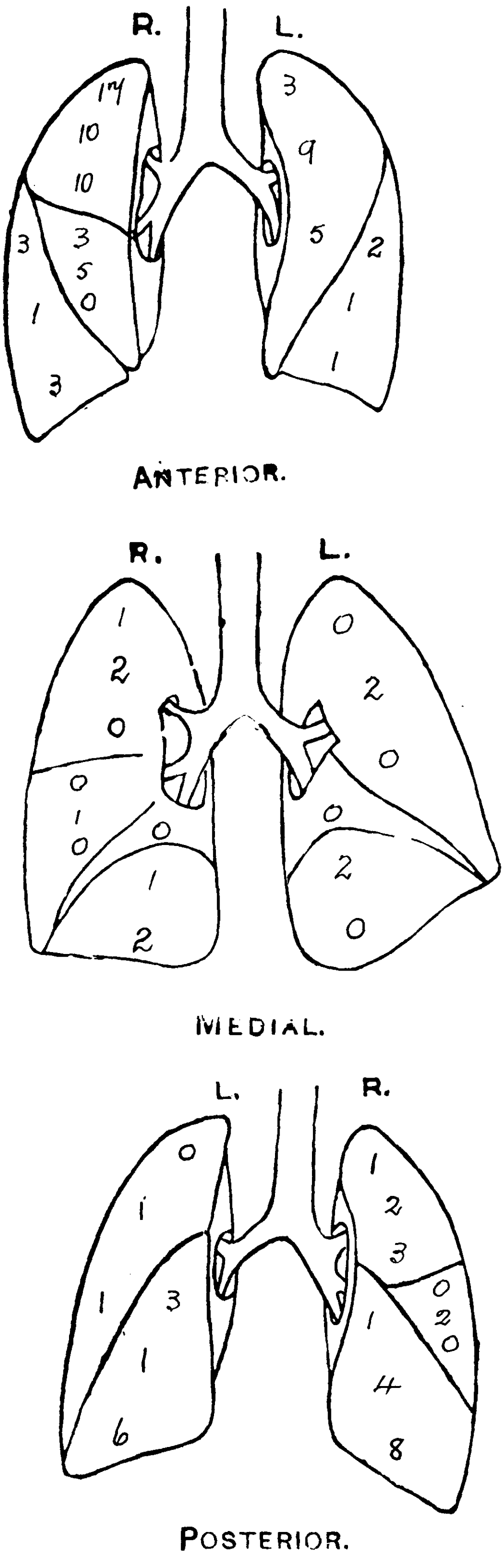


Fig. 11. Distribution of the 117 primary foci according to the aspects of the various lobes in which they were found, and also the position in these aspects in relation to upper, middle, and lower thirds. (Compare table XV.).

/6135

Fig. 12.

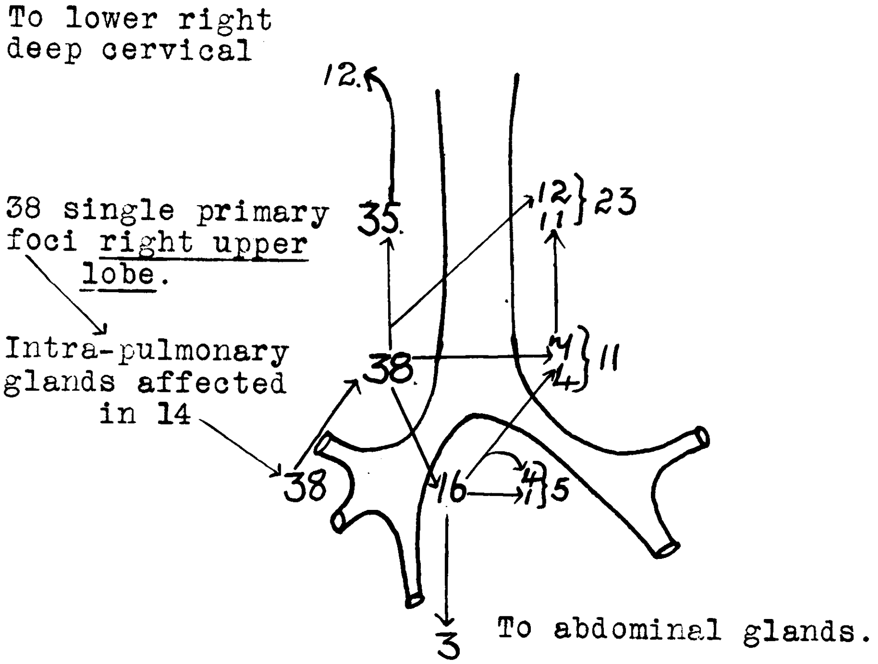


Fig. 12. The distribution of the tuberculous lesions in the tracheo-bronchial lymph nodes in 38 cases with one focus each in right upper lobe.

38 cases
- 3 cases (right broncho-pulmonary) (right superior tracheo-bronchial) only involved.
- 35 cases - the above glands in addition to others.

Downward spread = 16 cases
- 11 cases (right superior) (right inferior) tracheo-bronchial
- 4* cases (right superior) (right inferior) (left inferior) (left superior) tracheo-bronchial
- 1 case (right superior) (right inferior) (left inferior) tracheo-bronchial

Spread across = 11 cases
- 7 cases (right superior) (left superior) tracheo-bronchial
- 4* cases (right superior) (left superior) (right inferior) (left inferior) tracheo-bronchial

Upward spread = 35 cases
- Direct = 12 right para-tracheal.
- Direct ~~and~~ across = 23 (right para-tracheal). (left para-tracheal).

*These cases are considered under two headings, as the spread of the disease may have taken place both directly across from the right superior group to the left and also from the left inferior upwards to the left superior.

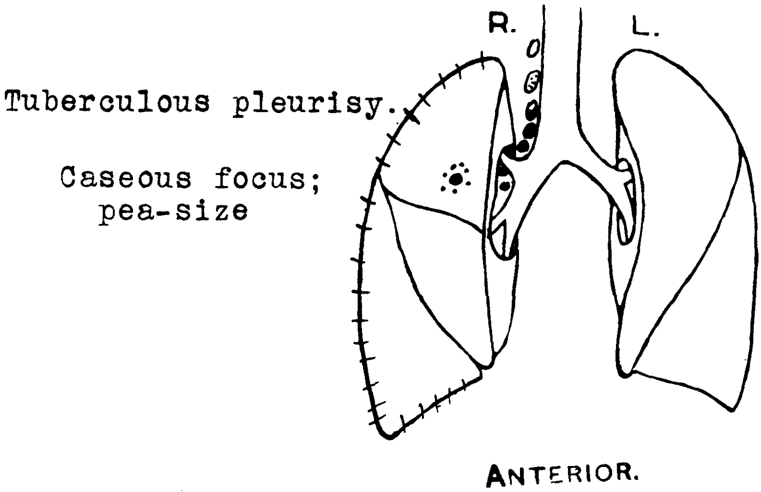


Fig. 13. Primary focus in right upper lobe with typical spread to superior tracheo-bronchial glands.

Case 1721. Male, aged 1 year, 1 month. No family history of tubercle.

Post-mortem. Lungs. Primary focus in lower third, anterior aspect of right upper lobe. Tuberculous caseous pleurisy on right side. Acute simple broncho-pneumonia.

Thoracic glands - See diagram. Only glands on right side involved, and only para-tracheal glands immediately above bifurcation.

Histology. Right pleura. Typical tuberculous pleurisy with caseation.

Bacteriology. Human strain isolated from right superior tracheo-bronchial glands.

Fig. 14.

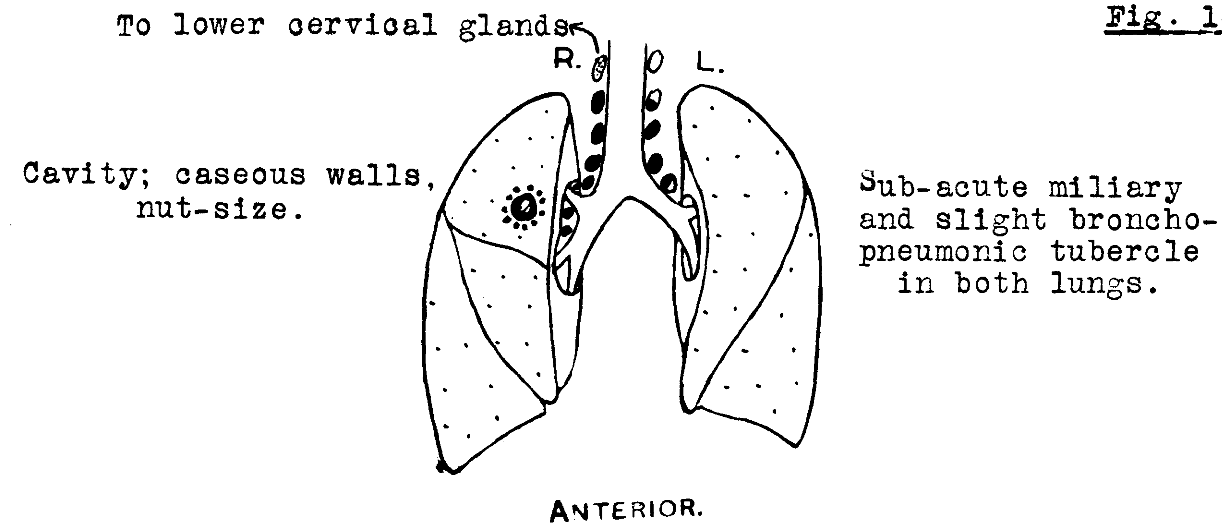


Fig. 14. Primary lesion in right upper lobe with involvement of superior tracheo-bronchial and para-tracheal on the opposite side.

Case 2614. Male, aged 8 months. No family history of tubercle.

Post-mortem. Lungs - See diagram.

Thoracic glands - See diagram. Lesion in right para-tracheal glands, diminished in severity on passing upwards. Few caseous foci in right lower cervical glands. On left side less severe and less extensive lesion in para-tracheal glands, and no extension to lower cervical as on right side.

Generalised sub-acute miliary tubercle; early tuberculous meningitis; tuberculous ulceration in upper part of small bowel.

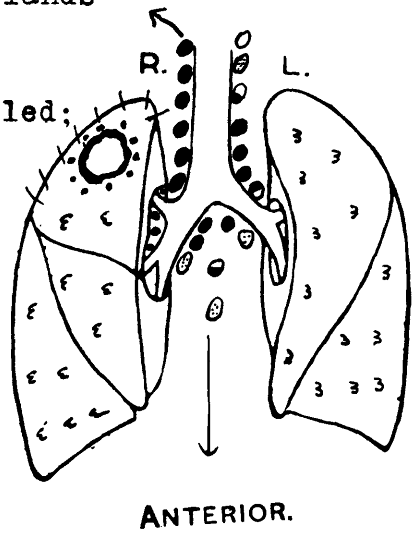
Histology. None.

Bacteriology. No organisms isolated.

Fig. 15.

To lower cervical glands

Cavity, caseous-walled; plum-size; fibrous pleurisy over upper lobe.



Extensive tuberculous broncho-pneumonia throughout both lungs.

Fig. 15. Primary focus in right upper lobe with extensive glandular involvement on both sides.

Case 2457. Male, aged 10 months. Family history - negative for tubercle.

Post-mortem. Lungs - See diagram.

Thoracic glands - See diagram. Right superior tracheo-bronchial glands most involved; some involvement of the right inferior tracheo-bronchial glands. Right para-tracheal glands markedly caseous, with spread to lower cervical glands. Left para-tracheal glands also involved, but not so marked as on the right side. Some of the pre-aortic glands in lower mediastinum showed caseous points.

Generalised miliary tuberculosis; extensive tuberculous ulceration of small and large bowel, with caseation of mesenteric glands.

Histology. None.

Bacteriology. Human strain isolated from right superior tracheo-bronchial glands.

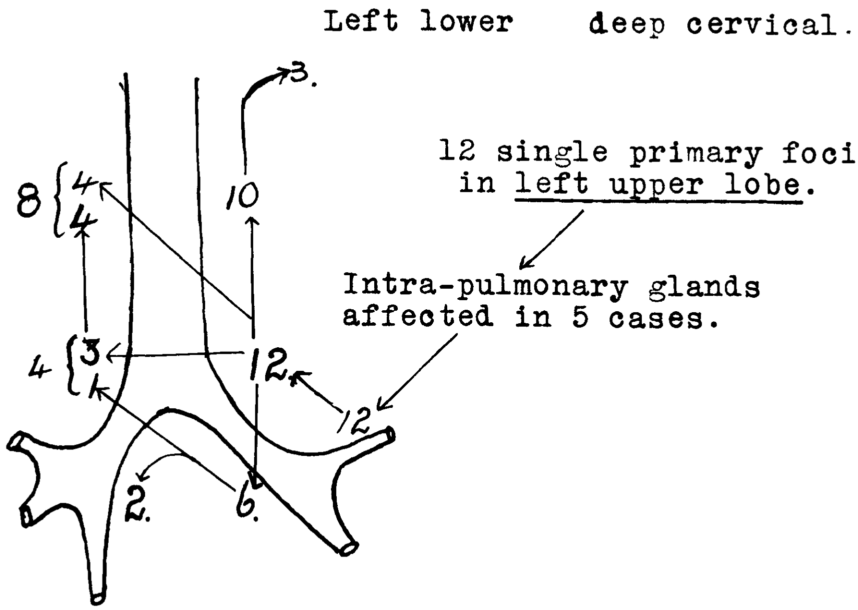


Fig. 16. The distribution of the tuberculous lesions in the tracheo-bronchial lymph nodes in 12 cases with one focus each in the left upper lobe.

12 cases:
- 2 cases (left broncho-pulmonary) only (left superior tracheo-bronchial) involved.
- 10 cases - the above glands in addition to others.

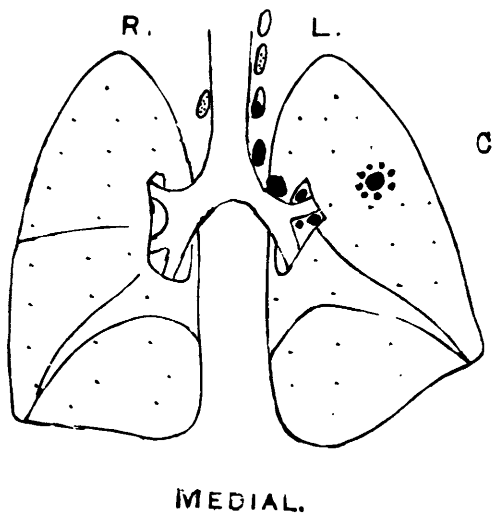
Downward spread - 6 cases:
- 4 cases (left superior) (left inferior) tracheo-bronchial.
- 1 case (left superior) (left inferior) (right inferior) tracheo-bronchial.
- 1 case* (left superior) (left inferior) (right inferior) (right superior) tracheo-bronchial.

Spread across - 4 cases:
- 3 cases (left superior) (right superior) tracheo-bronchial.
- 1 case* (left superior) (right superior) (left inferior) (right inferior) tracheo-bronchial.

Upward spread - 10 cases:
- Direct - 2 left para-tracheal.
- Direct and across. - 8 (left para-tracheal). (right para-tracheal).

* This case is considered in two sections as spread may have taken place either across from one set of superior glands to the other, or upward from the inferior.

Sub-acute miliary tubercles throughout both lungs.



Caseous focus; nut-size.

Fig. 17. Primary focus in left upper lobe with typical spread of the tuberculous disease to the left tracheo-bronchial lymph nodes.

Case 2167. Male, aged 2 years, 9 months. Mother died 4 weeks before with pulmonary phthisis.

Post-mortem. Lungs - See diagram.

Thoracic glands - See diagram. The only gland involved on the right side was one of the right para-tracheal, which showed a few caseous points.

Slight generalised miliary tuberculosis; no meningitis.

Histology. None.

Bacteriology. Human strain isolated from primary focus.

Fig. 18.

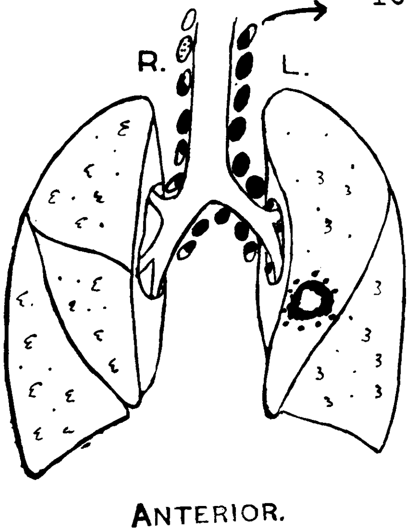


Fig. 18. Primary focus in left upper lobe with extensive spread of the tuberculous disease to the tracheo-bronchial glands on both sides.

Case 2344. Female, aged 18 months. Step-sister suffering from pulmonary tubercle.

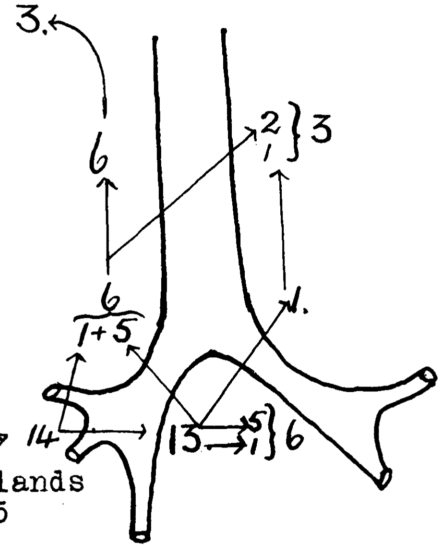
Post-mortem. Lungs - See diagram.

Thoracic glands - Very extensive involvement of left tracheo-bronchial glands, the lesions being most marked in left upper tracheo-bronchial. Glands on right side also involved, but to a less degree than on left.

Extensive tuberculous ulceration of small bowel (sputum infection) with caseous mesenteric glands. Tuberculous meningitis. Tuberculous disease of middle ears on both sides.

Bacteriology. Human strain isolated from left superior tracheo-bronchial glands.

To lower deep cervical on right side.



Intra-pulmonary glands affected in 5

14 single primary foci in right lower lobe.

Fig.19. The distribution of the tuberculous lesions in the tracheo-bronchial glands in 14 cases with one primary focus each in right lower lobe.

14 cases:

- 7 cases (right broncho-pulmonary) (right inferior tracheo-bronchial) only involved.
- 6 cases - the above glands in addition to others.
- 1 case (right broncho-pulmonary) (right superior tracheo-bronchial) (No lesion in inferior tracheo-bronchial.) in addition to others.

Tracheo-bronchial glands.

Upward spread = 5 cases

- 4 cases (right inferior) (right superior) (left inferior)
- 1 case (right inferior) (right superior) (left inferior) (left superior)

Spread across = 6 cases

- 5 cases already considered in upward spread.
- 1 case (right inferior) (left inferior) tracheo-bronchial.

Para-tracheal glands.

Upward spread = 6 cases

- Direct = 3 right para-tracheal.
- Direct and across = 3 (right para-tracheal) (left para-tracheal)

Fig. 20.

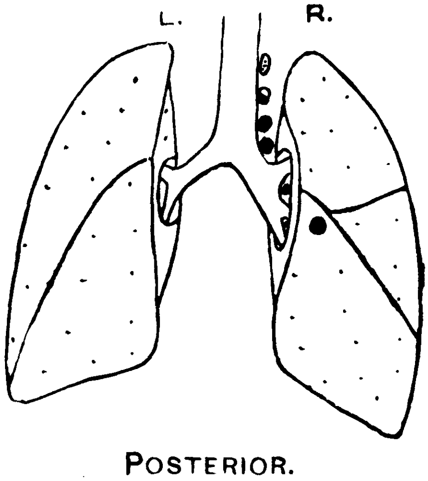


Fig.20. Primary focus in right lower lobe; no involvement of right inferior tracheo-bronchial glands, but right superior were found to be completely caseous.

Case 2782. Female, aged 3 years. Mother suffered from pulmonary consumption; other two children suffer from tuberculous disease; in the one the peritoneum was involved, and in the other the joints.

Post-mortem. Lungs - See diagram.

Thoracic glands - See diagram. No lesion found in the right inferior tracheo-bronchial.

Generalised sub-acute miliary tuberculosis; tuberculous meningitis.

Histology. Primary focus was caseous; secondary tubercles around, and early fibrosis; no calcification.

Bacteriology. Human strain isolated from right superior tracheo-bronchial lymph nodes.

Fig. 21.

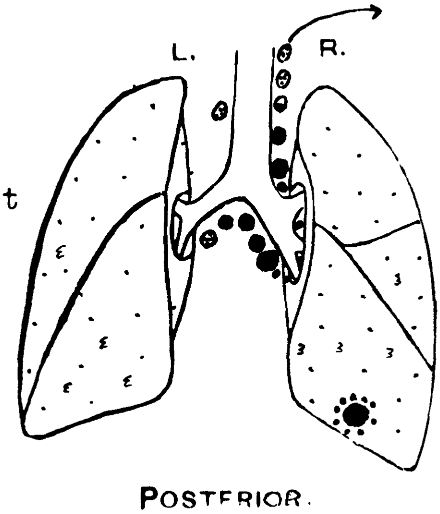


Fig. 21. **Primary focus in right lower lobe with typical spread of the tuberculous disease to the tracheo-bronchial glands.**

Case 1925. Female, aged 4 months. No family history of tubercle.

Post-mortem. Lungs - See diagram.

Thoracic glands - See diagram.

Generalised sub-acute miliary tuberculosis; scanty tuberculous ulcers in duodenum and upper part of jejunum (sputum infection); mesenteric glands in relation slightly caseous. Tuberculous meningitis.

Histology. Primary focus; caseous with softening and early cavitation; secondary tubercles and fibrosis around; no calcification. (See fig. 31).

Bacteriology. Human strain isolated from right inferior tracheo-bronchial glands.

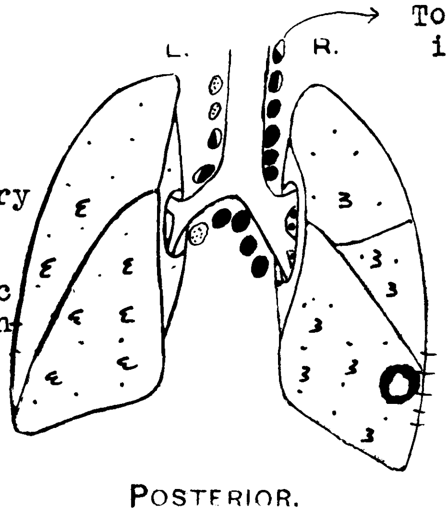


Fig. 22. Primary focus in right lower lobe with extensive tuberculous lesions in tracheo-bronchial glands.

Case 2633. Male, aged 5 months. No family history of tubercle.

Post-mortem. Lungs - See diagram.

Thoracic glands - See diagram. The glands on the left side show less involvement than on the right.

Generalised miliary tuberculosis; tuberculous meningitis; multiple tuberculous ulcers in upper half of small bowel (sputum infection); mesenteric glands caseous in relation to ulcers.

Histology. Caseous-walled cavity; no fibrosis; no calcification; abundant tubercle bacilli.

Bacteriology. Human strain isolated from right inferior tracheo-bronchial lymph nodes.

Fig. 23.

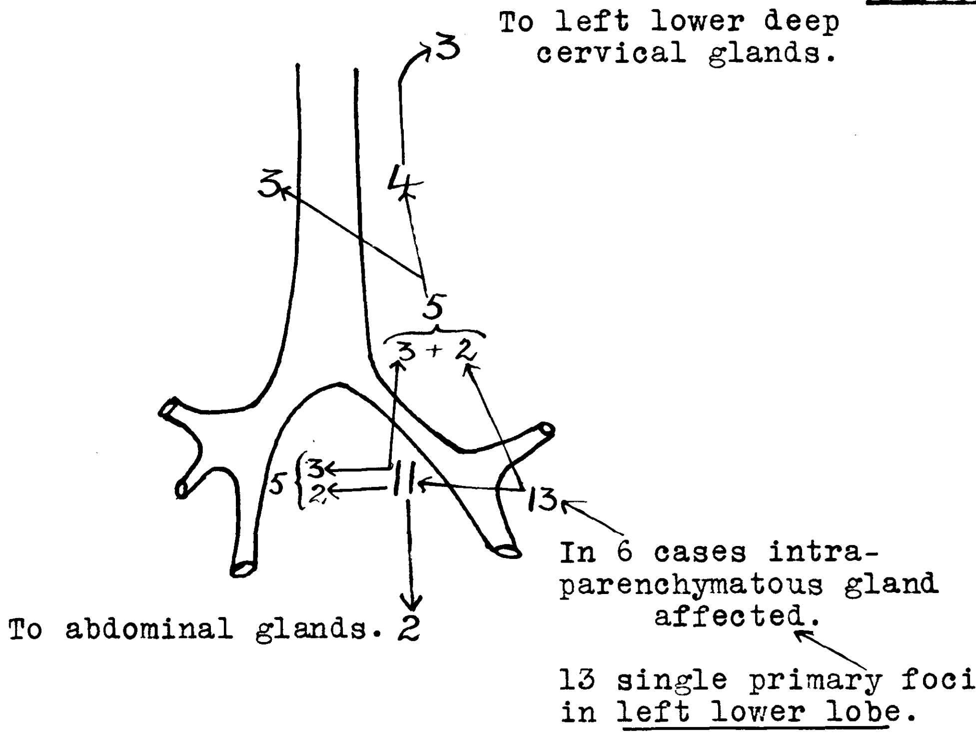


Fig. 23. <u>The distribution of the tuberculous lesions in the tracheo-bronchial lymph nodes in 13 cases with one focus each in the left lower lobe.</u>

<u>13 cases</u>

- 6 cases (left broncho-pulmonary / left inferior tracheo-bronchial) only involved.
- 5 cases - the above glands in addition to others.
- 2 cases (left broncho-pulmonary / left <u>superior</u> tracheo-bronchial. / <u>No lesion</u> in <u>inferior</u> tracheo-bronchial.) in addition to others.

<u>Tracheo-bronchial glands.</u>

Upward spread - <u>3 cases</u> (left inferior / right inferior / left superior) tracheo-bronchial.

Spread across - <u>5 cases</u> (3 cases already considered in upward spread. / 2 cases (left inferior / right inferior) tracheo-bronchial.)

<u>Para-tracheal glands.</u>

Upward spread
- <u>Direct</u> - 1 left para-tracheal.
- <u>Direct and across.</u> - 3 (left para-tracheal. / right para-tracheal)

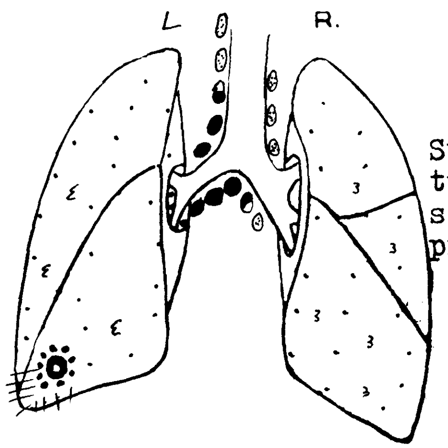


Fig. 24. Primary focus in left lower lobe with typical (though rather extensive) tuberculous lesions in tracheo-bronchial lymph nodes.

Case 2162. Female, aged 8 months. Mother suffering from phthisis.

Post-mortem. Lungs - See diagram.

Thoracic glands - See diagram. Those on the left side, particularly the left inferior tracheo-bronchial, involved to the greatest extent.

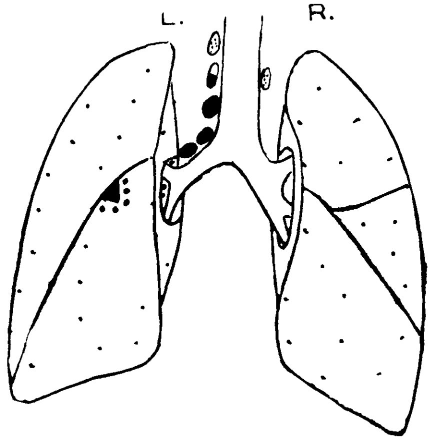
Generalised sub-acute miliary tuberculosis; tuberculous mass (tuberculoma) in brain; few tuberculous ulcers in upper part of jejunum, and mesenteric glands in relation to these caseous.

Histology. Caseous-walled cavity; no fibrosis; no calcification. The pleura showed fibrous thickening over the site of the cavity.

Bacteriology. Human strain isolated from left inferior tracheo-bronchial lymph nodes.

Fig/ 25.

Caseous focus - nut-size; sub-pleural.



Sub-acute miliary tubercles throughout both lungs.

POSTERIOR.

Fig. 25. Primary focus in left lower lobe; no involvement of corresponding inferior tracheo-bronchial glands. Right superior tracheo-bronchial completely caseous.

Case 2300. Male, aged 8 months. No family history of tubercle.

Post-mortem. Lungs - See diagram.

Thoracic glands - No lesion present in left inferior tracheo-bronchial, though left superior extensively tuberculous.

Slight generalised miliary tuberculosis; no meningitis. One of left para-tracheal glands adherent to oesophagus and ulcerated into it; scanty tuberculous ulcers in upper part of small intestine; mesenteric glands in relation to these slightly caseous.

Histology. Primary focus caseous; no softening; secondary tubercles around.

Bacteriology. Human strain isolated from the left superior tracheo-bronchial glands.

Fig. 26.

The distribution of the tuberculous lesions in the tracheo-bronchial glands in 7 cases with one primary focus each in the right middle lobe.

7 cases	1 case	(right broncho-pulmonary) (right inferior tracheo-bronchial)	only involved.
	5 cases	- the above in addition to others.	
	1 case	(right broncho-pulmonary) (right superior tracheo-bronchial) (No lesion in inferior tracheo-bronchial)	in addition to others.

Tracheo-bronchial glands.

Spread upwards - 4 cases	3	(right inferior) (right superior)	tracheo-bronchial.
	1 *	(right inferior) (right superior) (left inferior)	tracheo-bronchial.
Spread across - 2 cases	1	(right inferior) (left inferior)	tracheo-bronchial.
	1 *	(right inferior) (left inferior) (right superior)	tracheo-bronchial.

Para-tracheal glands.

Spread upwards - 5 cases	3	- right para-tracheal
	2	(right para-tracheal) (left para-tracheal)

* This is the same case, and has been considered under two routes of spread.

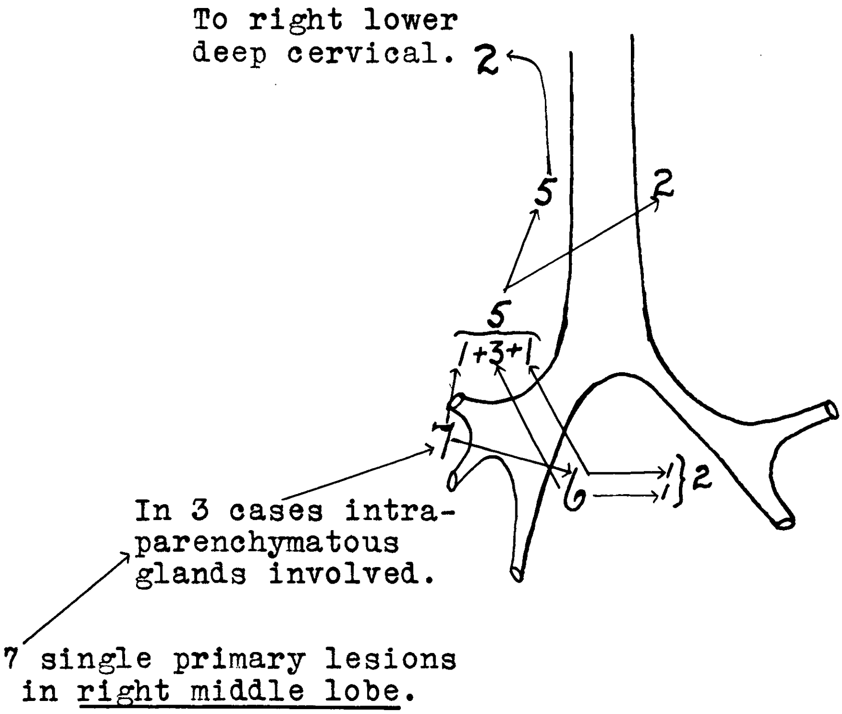
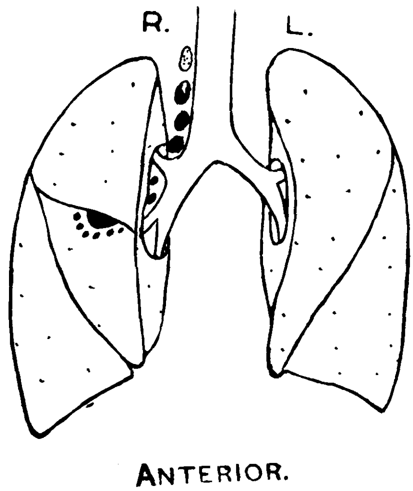


Fig. 26. The distribution of the tuberculous lesions in the tracheo-bronchial glands in 7 cases with one primary focus each in the right middle lobe.

Pea-sized caseous area; sub-pleural.



Sub-acute miliary tubercles throughout both lungs.

Fig. 27. Primary focus in right middle lobe; no tuberculous lesion found in right inferior tracheo-bronchial glands. Right superior tracheo-bronchial showed extensive tuberculous disease.

Case 2611. Female, aged 2 years. No family history of tubercle.

Post-mortem. Lungs - See diagram.

Thoracic glands - No lesion in right inferior tracheo-bronchial; right superior completely caseous.

Generalised miliary tuberculosis; tuberculous meningitis.

Histology. None.

Bacteriology. Human strain isolated from primary focus.

Fibrous pleurisy; adhesions.

Caseous focus - sub-pleural; nut-size.

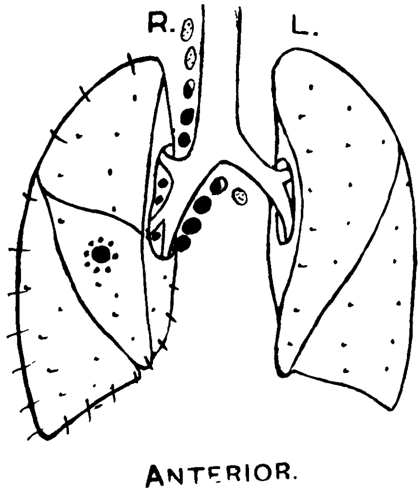


Fig. 28. Primary focus in right middle lobe with typical spread of the tuberculous disease to the tracheo-bronchial glands.

Case 2401. Female, aged 6 months. No family history of tubercle.

Post-mortem. Lungs - See diagram.

Thoracic glands - Right inferior tracheo-bronchial most involved; no lesion in glands on left side.

Generalised sub-acute miliary tuberculosis; tuberculous meningitis; tuberculomata.

Histology. None.

Bacteriology. Human strain isolated from right inferior tracheo-bronchial gland.

Fig. 29a. Caseous sub-pleural lesion due to a secondary vascular involvement in a case with primary lung focus. The almost closed vessel, in the wall of which a miliary tubercle is present, is seen just under the centre of the roughly wedge-shaped caseous area. Haematoxylin and eosin. x $6\frac{1}{2}$.

Fig. 29b. A higher power view of the above to show the endarteritis obliterans in the vessel. Haematoxylin and eosin. x 26.

Fig. 30. A bronchiole plugged with caseous debris leading into a primary lung focus which is seen above and to the right. At the edge of the caseous material in the focus there is marked cellular reaction with the formation of giant cells and early fibrosis. Haematoxylin and eosin. x 60.

Fig. 29a.

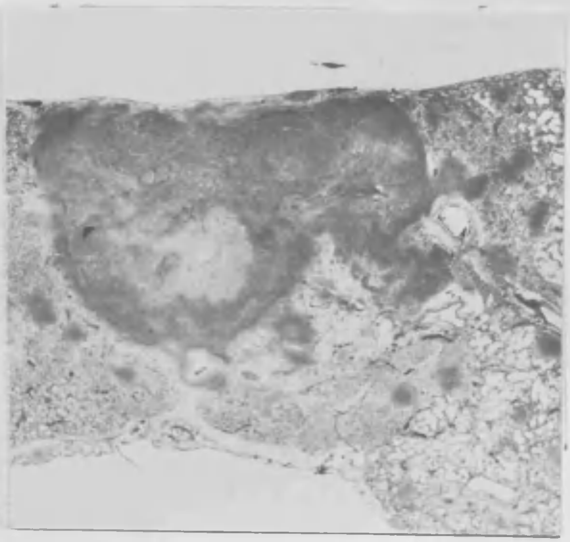
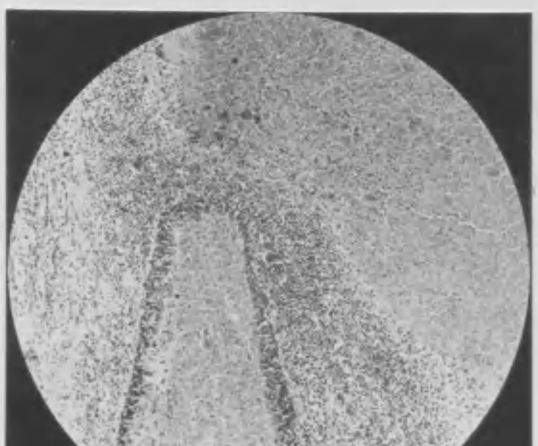


Fig. 29b.

Fig. 30.



Lesions from youngest cases showing fibrosis and calcification in tuberculous lesions.

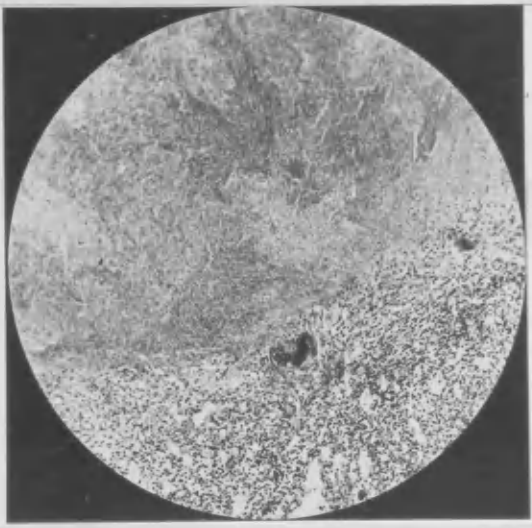


Fig. 31. Section from the edge of a primary focus from the youngest case (4 months) in which fibrosis at the edge was observed microscopically. The caseous material of the focus is seen above, and at the edge there is marked infiltration of cells with the formation of giant cells and young fibrous tissue.
Haematoxylin and eosin x 75.

Fig. 32. Section from tracheo-bronchial gland from youngest case ($6\frac{1}{2}$ months) in which deposition of lime salts was found histologically. The darkly staining crescentic mass in the caseous material in the gland is due to an early deposit of calcium salts which has been stained by von Kossa's silver method. x $6\frac{1}{2}$.

Sections from lung focus at an early stage.

Fig. 33a. The earliest histological stage at which the primary lung lesion was found in the investigation. The lesion, which is situated immediately under the pleura, is seen to be fairly sharply marked off from the surrounding lung tissue which shows little morbid change. The central part of the lesion is caseous and shows early softening. Numerous small vessels are involved in the lesion. The smaller tubercles in the lower part of the figure are secondary sub-acute miliary lesions. Haematoxylin and eosin. x 6½.

Fig. 33b. A higher power view of the edge of the caseous centre of the above lesion showing the cellular reaction in the air vesicles and the formation of endothelioid and giant cells and young fibrous tissue cells. The caseous material is above. Haematoxylin and eosin. x 140.

Fig. 33c. A high power to show the abundant tubercle bacilli at the edge of the lesion. Ziehl Neelsen. x 300.

Fig. 33d. The caseous centre of the lesion where early breaking-down is present to show the bacilli which, though numerous, are not so abundant as at the edge. Ziehl Neelsen. x 300.

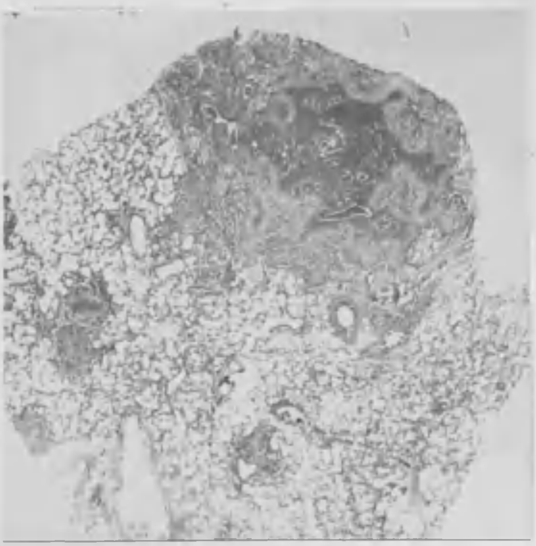


Fig. 33a.

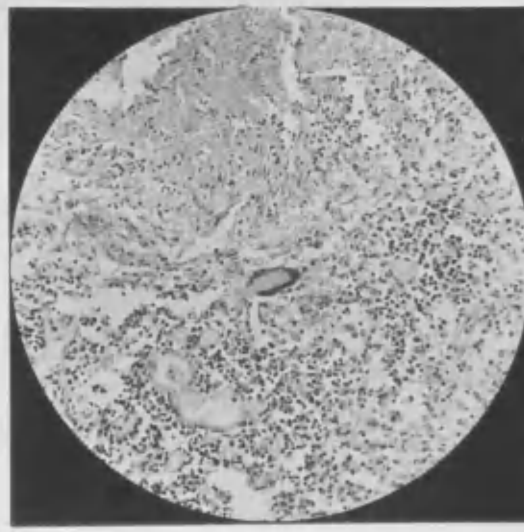


Fig. 33b.

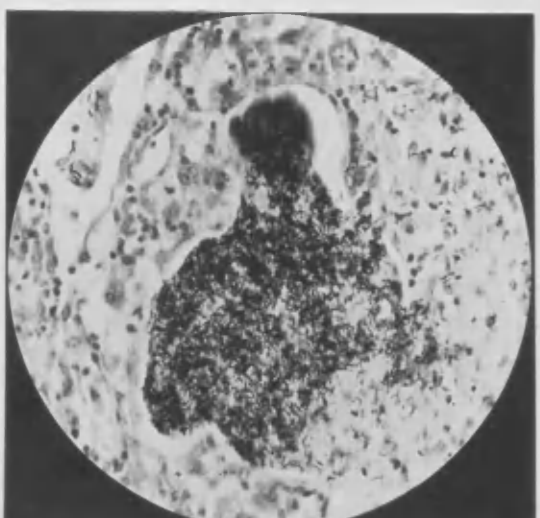


Fig. 33c.

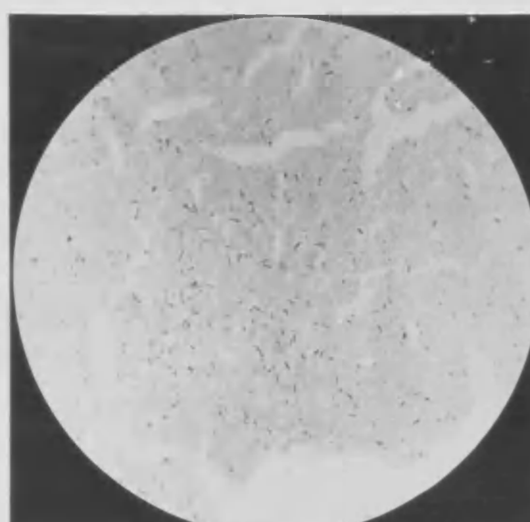


Fig. 33d.

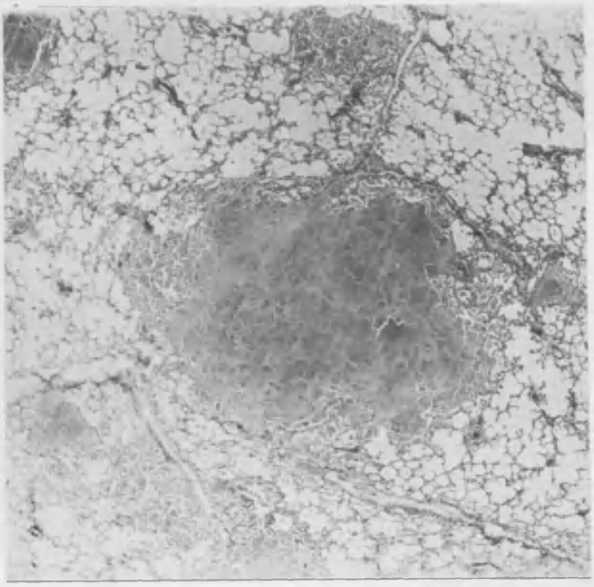


Fig. 34. A typical patch of secondary tuberculous broncho-pneumonia in a case with cavity formation showing the ill-defined edge, massive caseation, and the relative absence of cellular reaction as compared with that in the primary lung focus. Haematoxylin and eosin x 15.

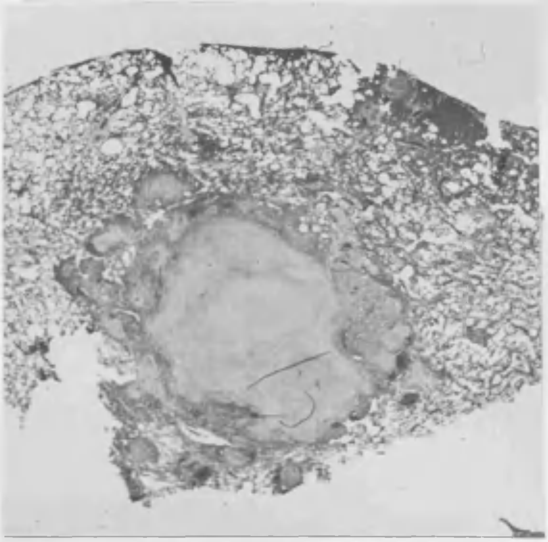


Fig. 35a. Completely caseous primary lung focus with secondary tubercles around. The lesion is situated a short distance under the pleural surface. Haematoxylin and eosin x $6\frac{1}{2}$.

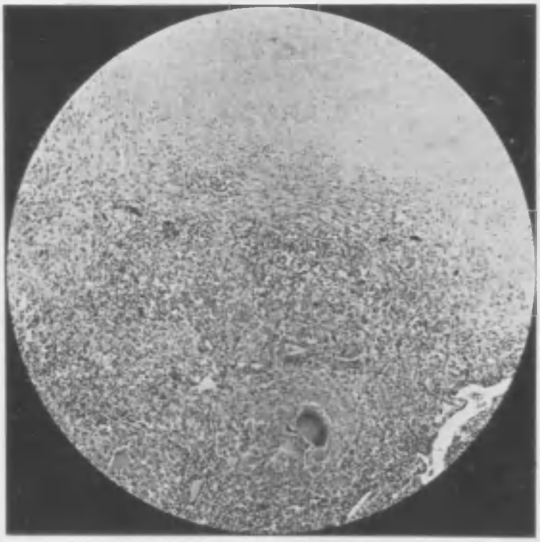


Fig. 35b. The edge of above showing the cellular infiltration, the well-formed tubercle follicles, and the encapsulating fibrous tissue at the edge of the caseous material. Haematoxylin and eosin x 70.

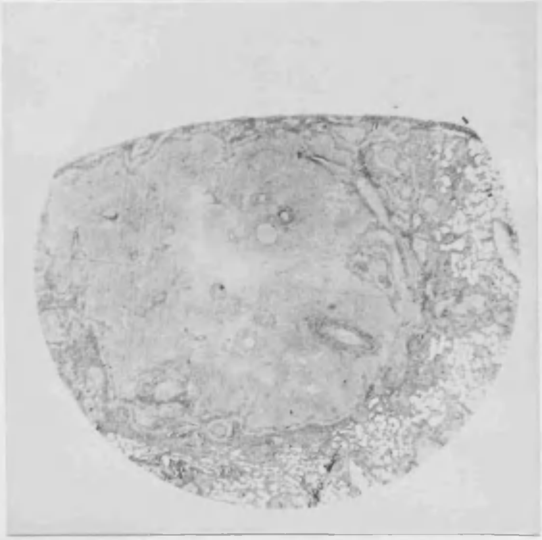


Fig. 36a. Completely caseous sub-pleural lung focus showing surrounding fibrosis and secondary tubercles. Several blood vessels are involved in the lesion. Gallego's modification of Mallory's stain x 5.

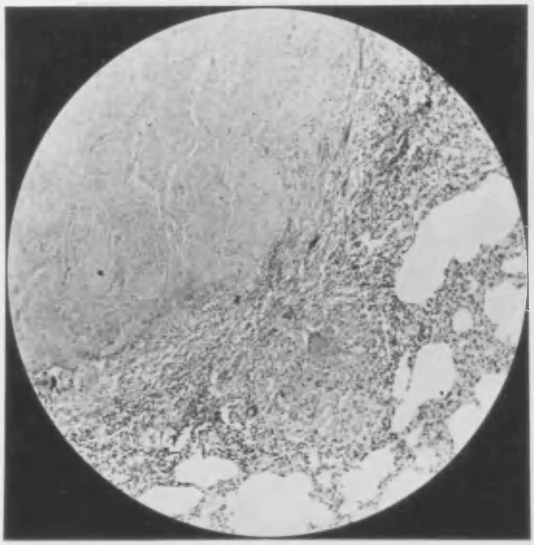


Fig. 36b. A higher power view of the edge of the above to show surrounding fibrosis and secondary tubercles. x 70.

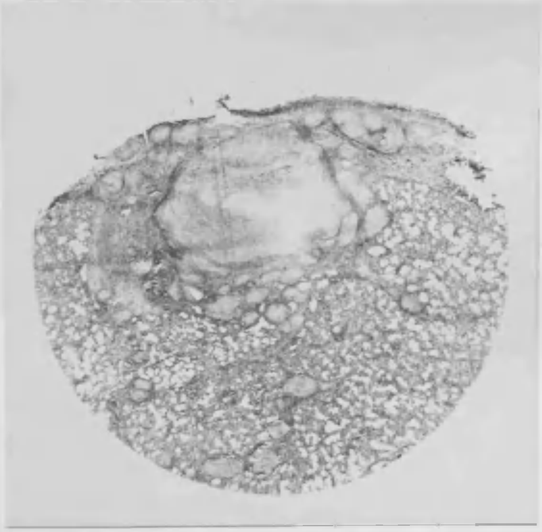


Fig. 37a. Caseous sub-pleural focus with well-marked surrounding fibrosis both at edge of focus and around the numerous secondary tubercles. The caseous material in the centre of the focus shows early softening. Gallego's modification of Mallory's stain x 5.

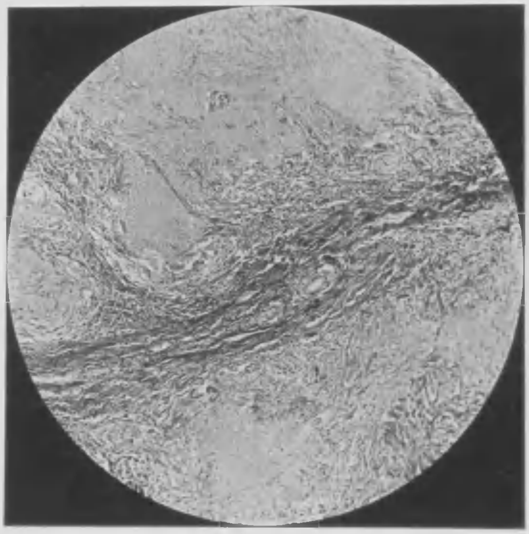


Fig. 37b. A higher power view of the edge of the above to show the dense fibrosis around the edge of the caseous material which is in the top part of the figure. Some secondary tubercles are seen below the fibrous tissue. x 70.

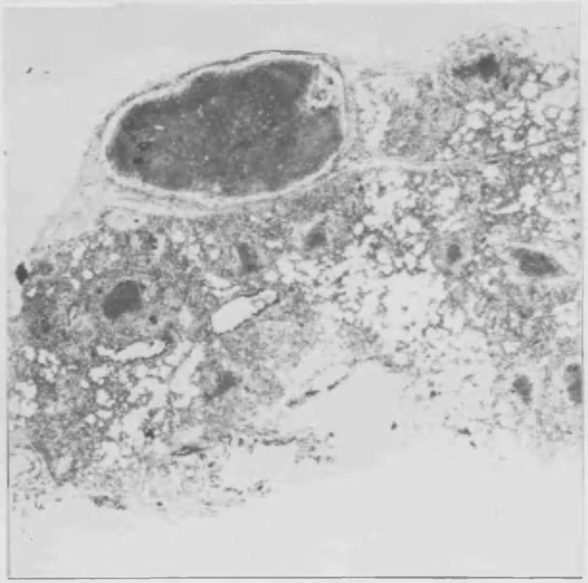


Fig. 38. Caseous sub-pleural lesion completely surrounded by fibrous tissue. The other lesions in the lower part of the figure are secondary sub-acute miliary and broncho-pneumonic tubercles. Gallego's modification of Mallory's stain x 10.

Sections from oldest primary lung lesion.

Fig. 39a. Section from the oldest lesion found showing the encapsulation with fibrous tissue and the absence of secondary tubercles. The central part is calcified and has fallen out in the preparation of the section. Some pleural thickening is present over the lesion, and the surrounding lung tissue shows only slight emphysema. Haematoxylin and van Gieson x $6\frac{1}{2}$.

Fig. 39b. The above stained by von Kossa's silver method to show deposit of calcium salts x $6\frac{1}{2}$.

Fig. 39c. A higher power view of the edge of fig. 39a to show the well-formed fibrous capsule around the lesion. The air vesicles at the edge show only slight emphysema. The caseous material is above and to left. x 75.

Fig. 39a.

Fig. 39b.

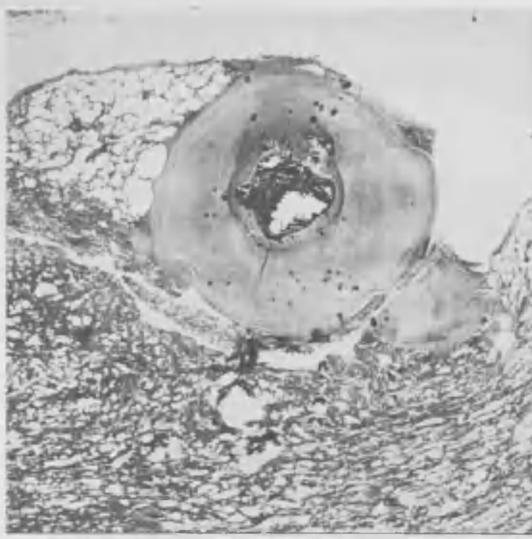
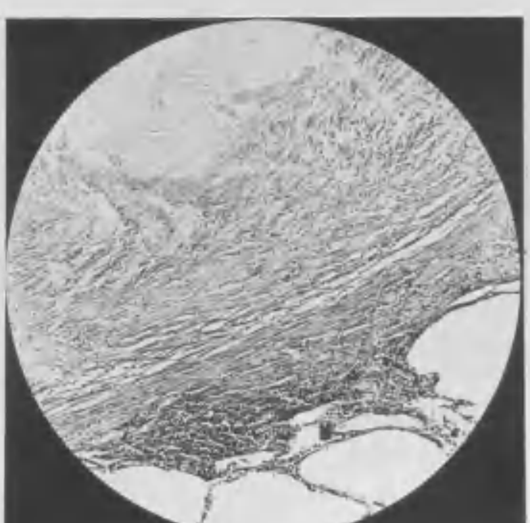


Fig. 39c.



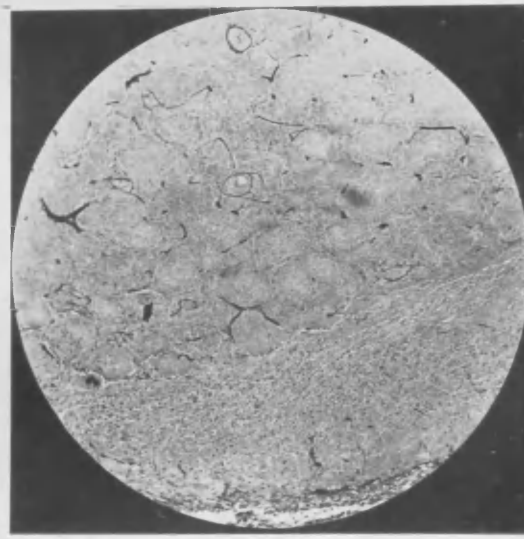


Fig. 40. Section from the primary focus shown in fig. 47a stained by Weigert's elastic tissue stain to show the normal arrangement of the elastic fibres of the air vesicles remaining in the caseous material which is at the top part of the figure and the encapsulating fibrous tissue below. x 60.

The interstitial change in the lung sometimes found associated with primary lung lesions.

Fig. 41a. Low power view of a primary lesion at the extreme base of the lower lobe. On two sides the lesion is limited by the pleura, while in the surrounding lung tissue there is much cellular infiltration. Haematoxylin and eosin x 10.

Fig. 41b. A higher power view of the edge of the above to show the type of cellular infiltration - lymphocytes and mononuclear cells with a few small giant cells. The fibrosis at the edge of the caseous material in the upper part of the figure is well seen. x 70.

Fig. 42. The deep surface of a sub-pleural lesion showing the encapsulation with fibrous tissue and also the great fibrous thickening of the trabeculae of the lung. The pleural surface of this lesion is shown in fig. 44. Haematoxylin and eosin x 12.

Fig. 41a.

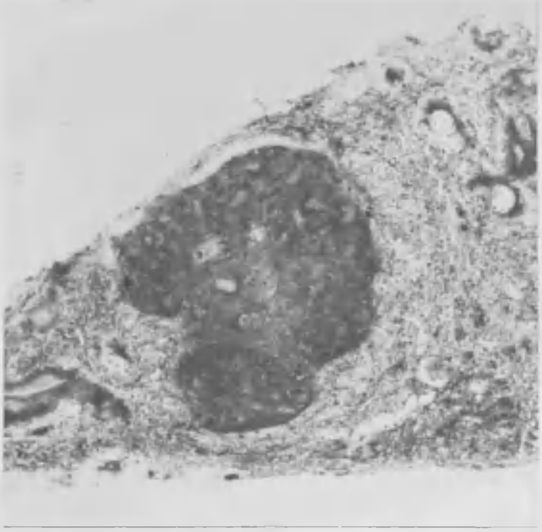


Fig. 41b.

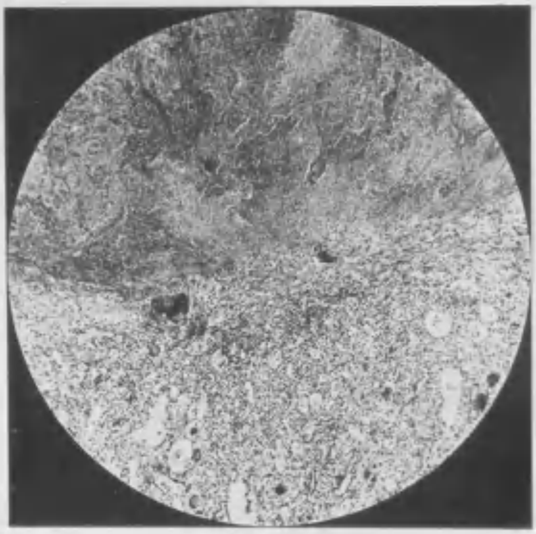
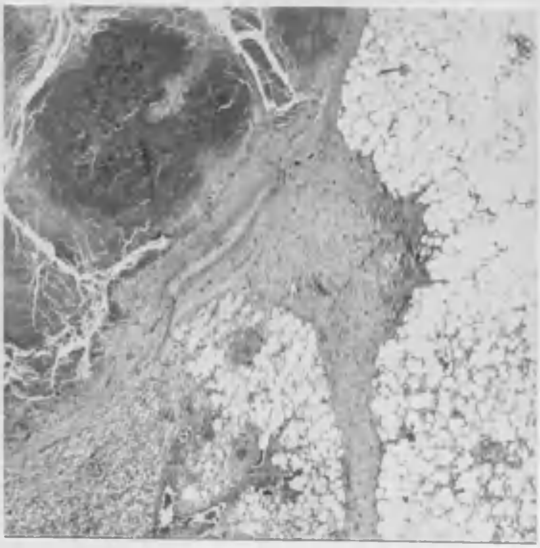


Fig. 42.



Pleural lesions associated with primary lung focus.

Fig. 43. Patch of caseous pleurisy near the site of a primary lung lesion, the end of which is seen as a small darkly staining triangular area below and to the left of the pleural lesion, which is well encapsulated. Haematoxylin and eosin x 6½.

Fig. 44. Great fibrous thickening of pleura over old primary lung lesion, the deep surface of which is shown in fig. 42. The caseous matter of the lung lesion is seen below, with much cellular reaction around on account of the early disintegration which is taking place. Haematoxylin and eosin x 60.

Fig. 45. Pleural adhesionsin vicinity of focus shown in fig. 37a. The tuberculous lesion has extended into the adhesions and the parietal pleura. Gallego's modification of Mallory's stain x 10.

Fig. 43.

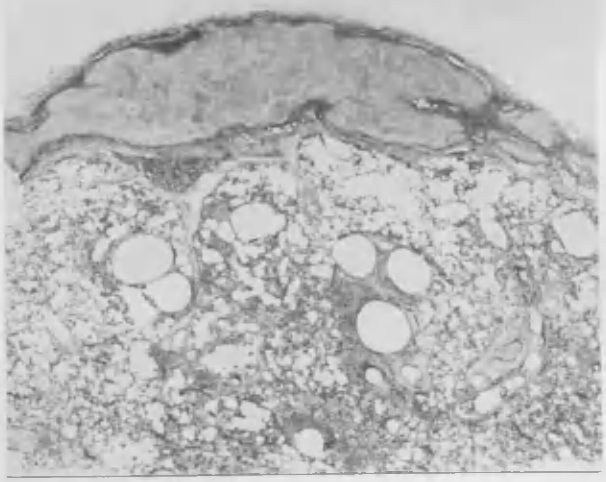


Fig. 44.

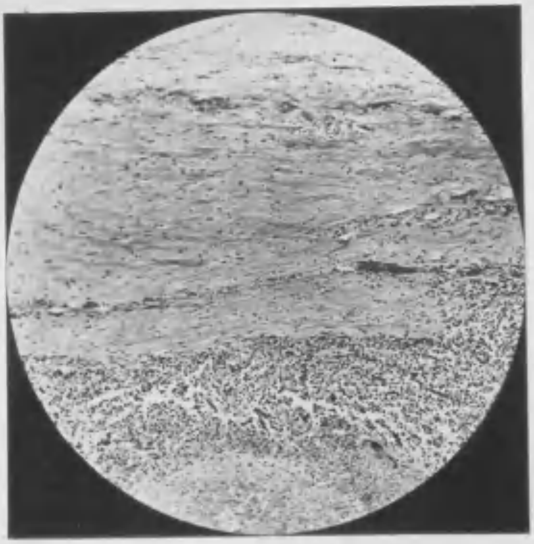
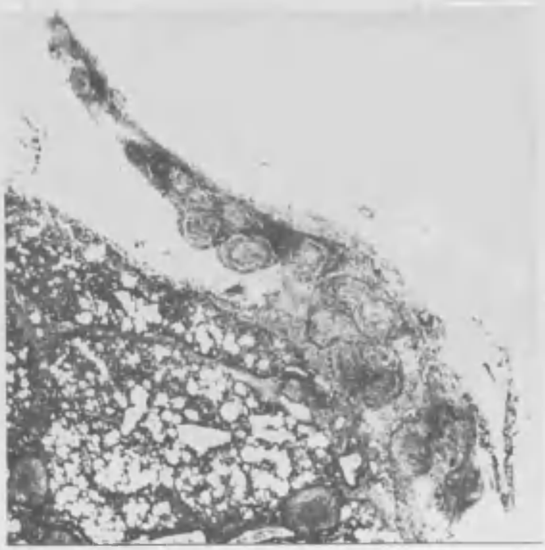


Fig. 45.



Sections showing the relation of the pathological lesion in the primary lung focus to that in the associated tracheo-bronchial glands.

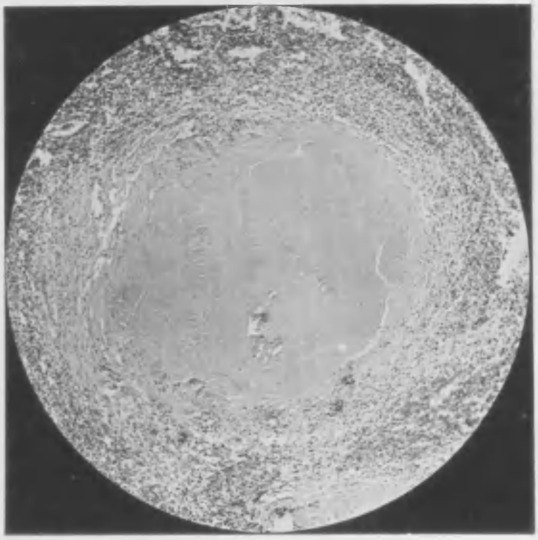


Fig. 46a. Section from an outgrowth of an irregular primary lesion to show caseation and surrounding fibrosis. For comparison with lesion in related tracheo-bronchial gland shown in fig. 46b. Haematoxylin and eosin. x 50.

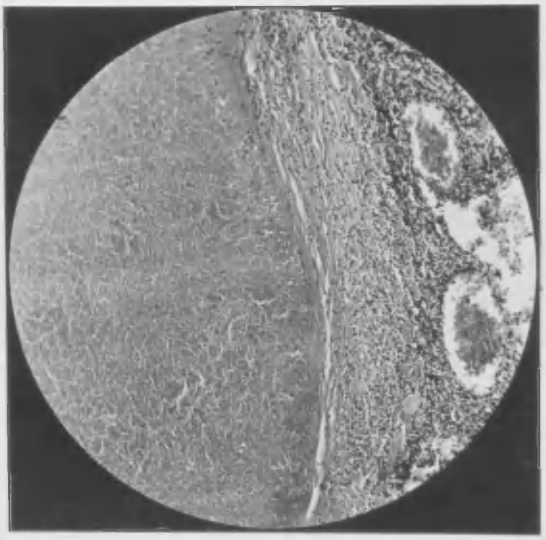


Fig. 46b. Section from one of the tracheo-bronchial glands related to the focus shown in fig. 46a. The surrounding fibrosis and cellular infiltration are similar to that present in the section from the primary lung lesion. Haematoxylin and eosin. x 75.

Sections showing the relation of the pathological lesion in the primary lung focus to that in the related tracheo-bronchial glands.

Fig. 47a. Section from primary focus found in substance of lung stained by haematoxylin. The presence of calcium salts is hardly shown; compare fig. 47b. x 5.

Fig. 47b. The above section stained by von Kossa's silver method. The presence of calcium salts is clearly demonstrated. x 6½.

Fig. 47c. Section from a tracheo-bronchial gland related to the focus shown in figs. 47a and 47b. Here, as in the primary focus, early deposit of calcium salts is present. Von Kossa's silver method x 6½.

Fig. 47a.

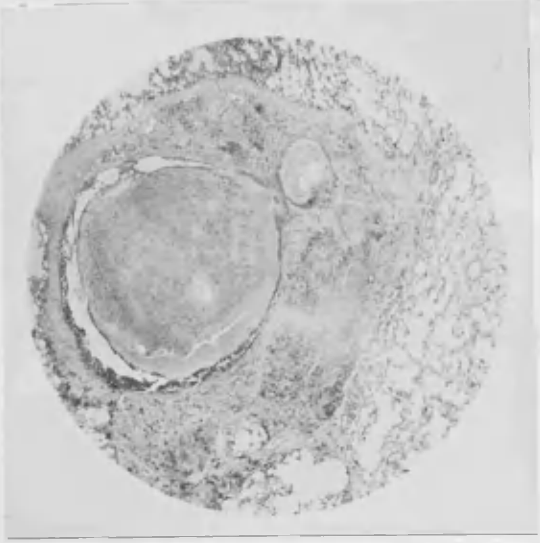


Fig. 47b.

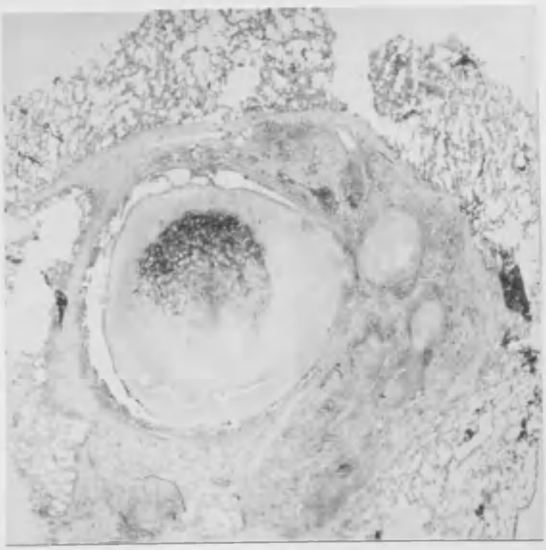


Fig. 47c.

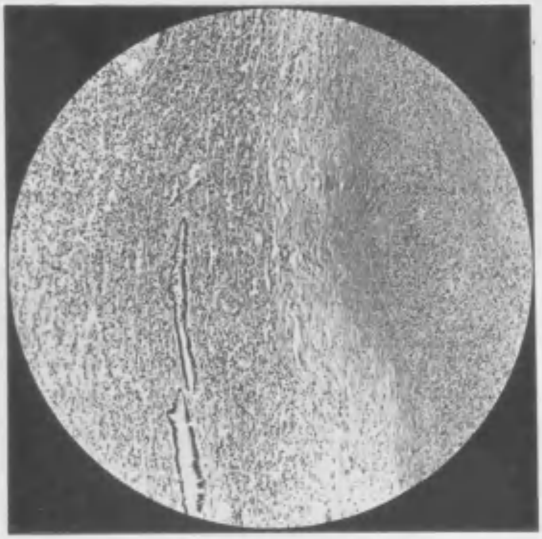


Fig. 48. Section from caseous lymph gland at hilum of lung. The interstitial change and collapse in the lung substance in the vicinity of the gland are well shown. The caseous material in the gland and fibrosis surrounding it are to the right. Haematoxylin and eosin. x 60.

Secondary lesions in lung in cases with primary lung foci.

Fig. 49. An early tuberculous broncho-pneumonic lesion. The bronchiole is plugged with cellular exudate which is undergoing caseous change, and there is much peri-bronchial cellular infiltration. Haematoxylin and eosin x 55.

Fig. 50. Section showing typical sub-acute miliary lesions in which there is central caseation and surrounding cellular reaction. Haematoxylin and eosin x $6\frac{1}{2}$.

Fig. 51. Section from a branch of the pulmonary vein in the intima of which there is a typical sub-acute miliary tubercle, the centre of which is caseous. Haematoxylin and eosin x 45.

Fig. 49.

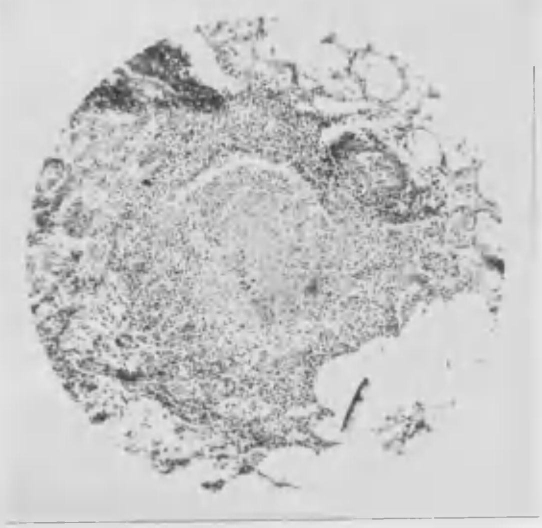


Fig. 50.

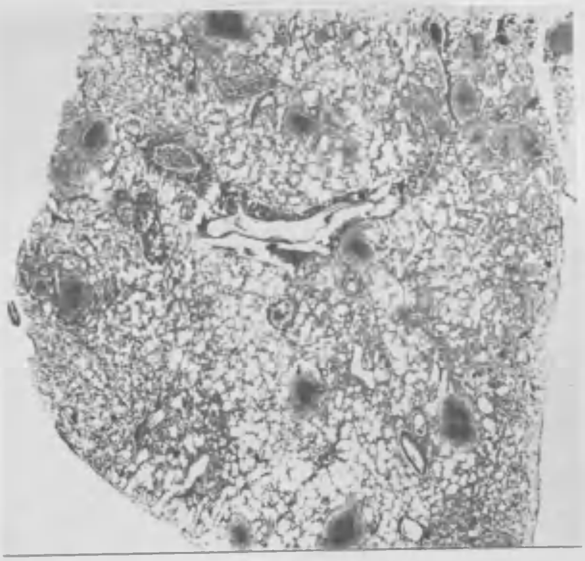


Fig. 51.

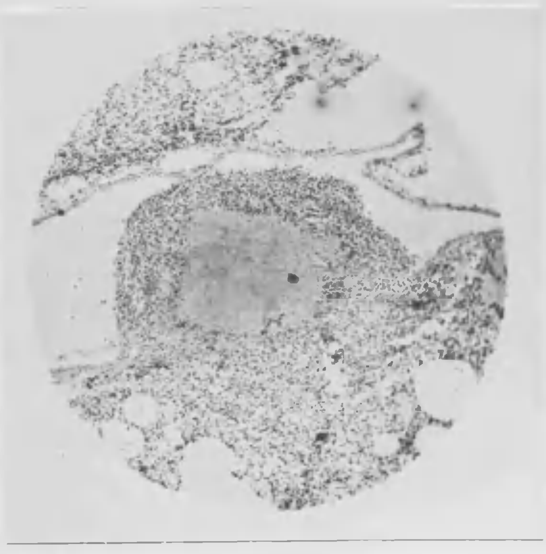
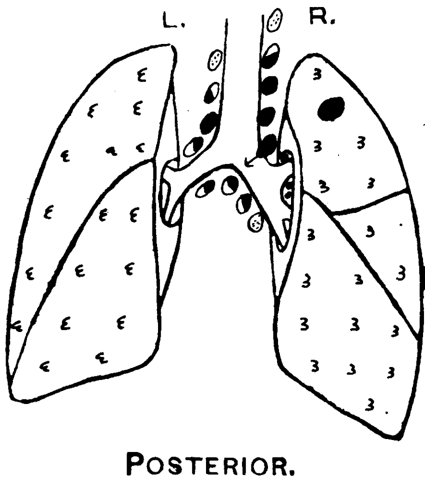


Fig. 52.

Widespread tuberculous broncho-pneumonia.



Caseous pea-sized, sub-pleural focus.

Fig. 52. Primary focus in right upper lobe; rupture of one of right superior tracheo-bronchial glands into right bronchus; extensive tuberculous broncho-pneumonia.

Case 1718. Female aged 1 year 5 months. No family history of tubercle.

Post-mortem. Lungs - See diagram. Primary focus in right upper lobe. Extensive tuberculous broncho-pneumonia with early cavitation.

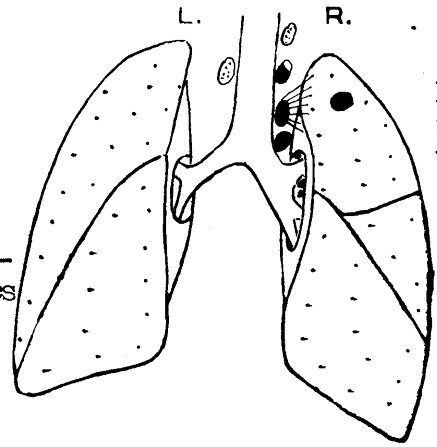
Thoracic glands. One of the caseous right superior tracheo-bronchial glands ruptured into right bronchus; chronic inflammatory thickening round site of rupture.

Generalised miliary tuberculosis; tuberculous ulceration of small bowel (sputum infection); caseous mesenteric glands.

Histology. Primary focus showed some fibrosis around; caseous material breaking down in centre. Tissue from other parts of lung showed acute tuberculous broncho-pneumonia.

Bacteriology. Human strain isolated from right superior tracheo-bronchial glands.

Fig.53.



Bean-sized caseous focus, short distance under pleural surface.

Sub-acute miliary and early bronchopneumonic tubercles throughout both lungs.

POSTERIOR.

<u>Fig. 53.</u> <u>Focus in right upper lobe; chronic adhesive pleurisy over related caseous right superior tracheo-bronchial gland.</u>

<u>Case 2351.</u> Female child aged 8 months. No family history of tubercle.

<u>Post-mortem.</u> <u>Lungs</u> - See diagram.

<u>Thoracic glands</u>. Chronic adhesive pleurisy in relation to one of right superior tracheo-bronchial glands. Generalised miliary tuberculosis; no meningitis.

<u>Bacteriology</u>. Human strain isolated from the caseous lung focus.

Fig. 54.

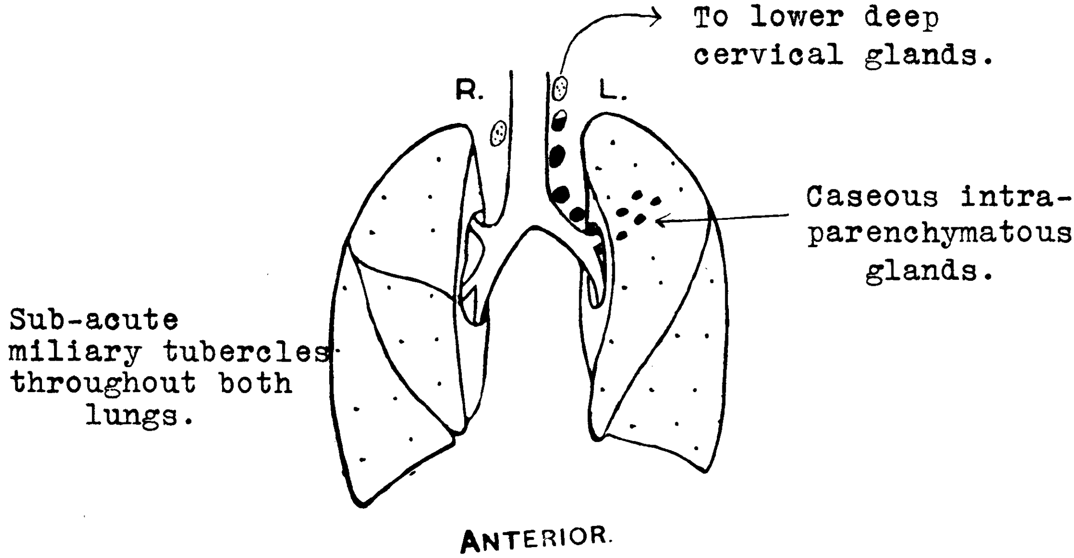


Fig. 54. Case in which no primary lung focus was found; caseous intra-parenchymatous glands in left upper lobe.

Case No. 2010. Male child aged 3 years 5 months. No family history of tubercle.

Post-mortem. Lungs - See diagram; no primary focus found.

Thoracic glands - See diagram.

Generalised miliary tuberculosis; tuberculous meningitis.

Bacteriology. Human strain isolated from left superior tracheo-bronchial glands.

Fig. 55. **Tuberculous right superior tracheo-bronchial glands due to infection with bovine virus.**

Case 2109. Male, aged 9 months. No family history of tubercle; breast-fed for 3 months and then cow's milk.

Post-mortem. Lungs - Slight bronchiectasis (verified histologically) in right upper lobe, slight acute pleurisy over this lobe. No primary lung focus found.

Thoracic glands - See diagram.

Generalised sub-acute miliary tuberculosis; tuberculous meningitis. No naked-eye lesion found in any of the abdominal or cervical lymphatic glands. The most advanced lesion appeared to be in right broncho-pulmonary and tracheo-bronchial glands.

Bacteriology. Abundant short bacilli in films made from antiformin sediment prepared from right superior tracheo-bronchial glands. Cultured direct a dysgonic growth resulted on egg, potato, and serum all of which contained glycerin from sub-cultures made from 4th transfer on ordinary egg. The cultures on glycerin potato and serum showed no pigmentation. Rabbit inoculated with 0.01 mg. of 3rd sub-culture died in 34 days with severe lung lesions and generalised miliary tuberculosis. Another rabbit similarly inoculated from 4th sub-culture died in 39 days with lesions corresponding to the first animal.

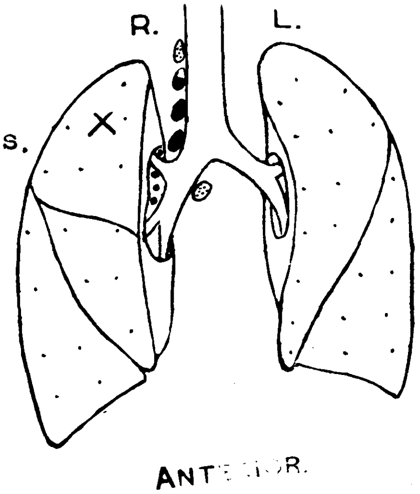


Fig. 55. Description on opposite page.

Fig. 56. Tuberculous right inferior tracheo-bronchial due to infection with bovine virus.

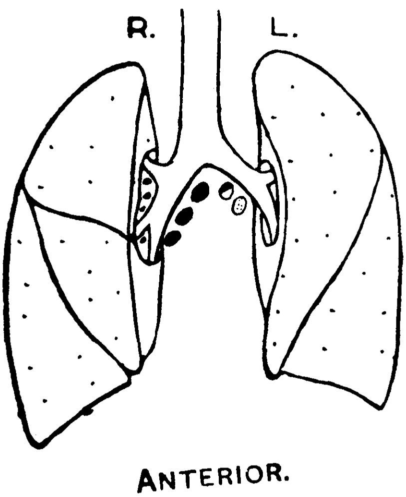
Case 2359. Female, aged 2 years, 3 months. No family history of tubercle; breast-fed for 1 year then general.

Post-mortem. Lungs - Sub-acute miliary tuberculosis no primary focus found.

Thoracic glands - Right inferior tracheo-bronchial glands caseous.

Generalised miliary tuberculosis; tuberculous meningitis. No naked-eye evidence of tuberculosis in mesenteric or cervical glands. The most advanced lesion was in the right inferior tracheo-bronchial glands.

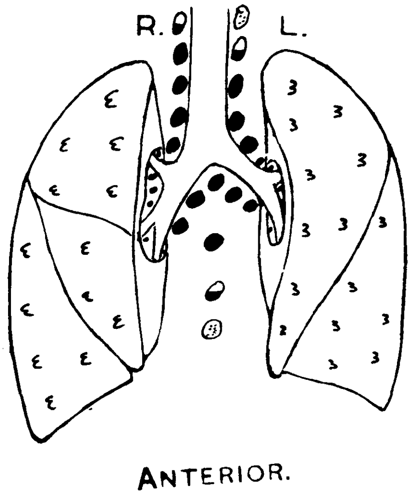
Bacteriology. Scanty medium-sized evenly staining rods in films of antiformin sediment prepared from right bronchial gland mass. Cultures isolated through guinea-pig proved dysgonic on egg, potato, and serum all of which contained glycerin in subcultures made from 2nd and 4th transfers on ordinary egg and there was no evidence of pigment production. Rabbit inoculated with 0.01 mg. of 2nd sub-culture died 36 days later with generalised miliary tuberculosis. Another rabbit inoculated from 4th sub-culture in a similar manner died 32 days later of generalised miliary tuberculosis and very extensive caseous lesions in lungs.



Sub-acute miliary tubercles in both lungs.

Fig. 56. Description on opposite page.

Fig. 57.



Marked confluent tuberculous broncho-pneumonia with cavitation.

Fig. 57. Case with severe tuberculous pulmonary lesions and extensive tuberculous adenitis of tracheo-bronchial glands; no primary focus found.

Case No. 1760. Female child aged 7 months. No family history of tubercle.

Post-mortem: Lungs. Widespread confluent caseous tuberculous broncho-pneumonia with cavitation.

Thoracic glands. All the groups of tracheo-bronchial glands involved as well as lymph nodes in the lower mediastinum.

Generalised miliary tuberculosis; tuberculous meningitis; multiple tuberculous ulcers throughout small bowel; extensive caseation of mesenteric glands.

Bacteriology. Human strain isolated from right superior tracheo-bronchial glands.

Fig. 58. **Case in which the most advanced tuberculous lesion was in the left paratracheal glands, from which a bovine strain was isolated.**

Case 1986. Male, aged 1 year, 6 months. No family history of tubercle; breast-fed 13 months and then general.

Post-mortem. Lungs - No primary lesion found sub-acute miliary tuberculosis.

Thoracic glands - One of left paratracheal glands completely caseous; caseous points in upper paratracheal glands and in lower deep cervical glands as well as in left superior tracheo-bronchial. No lesion in left broncho-pulmonary glands.

Generalised miliary tuberculosis; tuberculous meningitis.

Bacteriology. Numerous short bacilli found in films of antiformin sediment prepared from left paratracheal glands. Cultured direct a dysgonic non-pigmented growth on glycerin egg and glycerin potato resulted from sub-culture made from 3rd and 4th transfers on ordinary egg. A rabbit inoculated with 0.01 mg. intravenously from 3rd sub-culture died of generalised miliary tuberculosis with marked lung lesions in 23 days, while another rabbit similarly inoculated from 4th sub-culture died in 27 days with similar lesions.

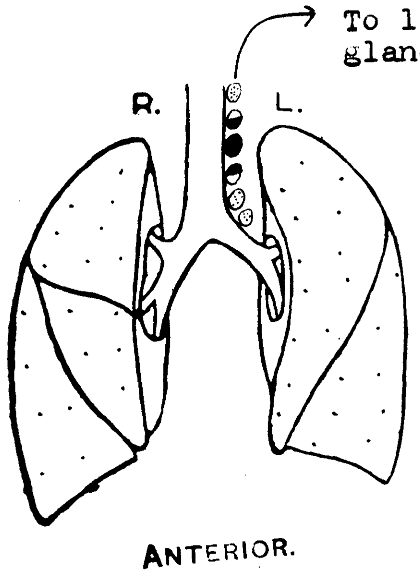


Fig. 58. Description on opposite page.