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A BACTERIOLOGICAL AND PATHOLOGICAL INVESTIGATION
OF TUBERCULOUS MENINGITIS
IN THE WESTERN PROVINCE OF THE CAPE OF GOOD HOPE

T H E S I S

Presented in part fulfilment of the requirements
for the degree of Doctor of Medicine in the De-
partment of Pathology.

BY

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INTRODUCTION

Although there have always been sporadic reports of spontaneous cures in tuberculous meningitis, up to a few years ago this diagnosis almost invariably meant death within a few weeks or months.

The therapeutic use of Streptomycin in this disease has created new hope for these patients and has stimulated tremendous interest in all aspects of the disease.

Some idea of the near epidemic proportions this disease assumes in the Cape Town area is obtained from the fact that for purposes of the present investigation 200 cases of tuberculous meningitis were collected in the span of 13 months, and when it is considered that this disease claims most of its victims in the first decade of life, the importance of the disease becomes manifest.

My introduction to the disease arose through a close association with the City Hospital for Infectious Diseases, where the sheer numbers of cases admitted demanded attention.

Interest in the bacteriology of tuberculous meningitis centres round two main problems, namely the epidemiology of the disease and the sensitivity of the causal organisms to Streptomycin.

Dealing with the second point first, pulmonary tuberculosis is now being extensively treated with Streptomycin and the possibility of meningeal infections occurring with initially resistant organisms is consequently greater than ever before and has been repeatedly stressed. In view of the indifferent results which were being obtained with Streptomycin in this disease, it was decided to establish at least this prerequisite for successful treatment, i.e. initial sensitivity to the drug, in every case investigated.

The/.....

The establishment of the epidemiology of the disease entails a knowledge of the types of infecting organisms, and this country has lagged far behind most others, especially the British Isles, in ascertaining the part played by the bovine tubercle bacillus in human disease. Certainly in the Western Province of the Cape no investigation of this nature has ever been reported, quite apart from any such attempt specifically directed towards tuberculous meningitis, and the establishment of the epidemiology of the disease in this area formed the second object of this investigation.

The idea that tuberculous disease in man was partly to be traced to infection from cattle had its beginning in the early part of the nineteenth century. Specific attempt at proof was first made by Klenke in 1846, who insisted on the infectiousness of milk from tuberculous cattle. The results of inoculation experiments culminating in the positive demonstration by Villemin in 1868 of the infectious nature of tuberculous material, from both man and cattle, for other animals, strengthened this view.

The first suggestion that human tuberculosis differed aetiologically from that which affects lower animals, is also to be found in the writings of this French military surgeon J.A. Villemin who published a series of epoch making papers on tuberculosis between the years 1865 and 1869, including a large book on the subject in 1868. Not only, as mentioned above, was he the first to prove the infectious nature of tuberculous material derived from both man and cattle for other animals, but he actually remarked "that none of our rabbits inoculated with human tuberculous material have presented a tuberculisation so rapid and generalised as that which we have obtained with material from the cow". This statement, which seems so significant in the light of present knowledge, was
 completely/.....

completely overlooked for at least two decades.

In March 1882, during an address to the Physiological Society of Berlin, Robert Koch announced the discovery of the tubercle bacillus, an organism which fulfilled all his postulates as being the cause of tuberculosis. He failed not only here, but also in his closely reasoned book on the aetiology of tuberculosis published in 1884, to mention any differences between the organisms of human and animal tuberculosis, and as a result of his cultural and animal experiments actually categorically denied that any such differences existed. It is extraordinary that such an acute observer as Koch undoubtedly was, should have missed the striking differences existing in the effects produced by injecting human and bovine bacilli respectively into various animals, and shortly after Koch's discovery in 1882, Klein and Gibbes (1883) in England pointed out that Koch's work had not clearly established the identity of the various types of bacilli, and actually performed experiments to prove differences in pathogenicity of tubercle bacilli derived from various sources.

Little attention was paid to Klein's work, and initially Koch also regarded the avian bacillus as being identical with the mammalian type. This mistake can possibly be explained by the fact that he had not been able to obtain cultures from fowls for want of fresh material, and basing his conclusions merely on morphological and staining similarities, believed himself, in spite of gaps in the evidence, to be in a position to speak of the essential identity of tuberculosis existing in the different species of animals.

He, however, quickly corrected his attitude with regard to avian tuberculosis when he received cultures

isolated/.....

isolated from tuberculous pheasants from two workers, Nocard and Roux, in Paris and he then immediately recognised that the differences existing between avian and mammalian tubercle bacilli were fundamental, and became convinced that the fowl tubercle bacillus belonged to a species distinct from, though closely related to, the mammalian type.

A little later (1889) two Italian workers, Rivolata and Maffucci, also differentiated the avian from the mammalian type.

The next step towards differentiation of the human and bovine types was taken in America by Theobald Smith who in 1896 published a comparative study of two strains of tubercle bacilli. The one strain was derived from a tuberculous bull, and the other from a pet animal believed to have contracted tuberculosis from its master. In these researches, which mark the commencement of modern views on the relationship of the bacilli of human and animal tuberculosis, decided differences in virulence for the ox and the rabbit of these two strains, one presumably human and the other bovine, came to light and morphological peculiarities were also described. It is an interesting fact to note that by examining these two cultures alone, Smith determined every essential difference existing between the two types of mammalian tubercle bacilli.

Two years later (1898) Smith recorded the results of investigations on additional strains of tubercle bacilli and suggested the existence of "a distinctive human or sputum, and a bovine variety of tubercle bacillus".

Generally, however, little attention was paid to Smith's discovery and the general belief in the identity of human and bovine tubercle bacilli remained undisturbed, or at most it was believed that tubercle bacilli from cattle

were/.....

were actually slightly more virulent for humans, and attempts at the eradication of bovine tuberculosis in dairy herds were in progress. Consequently the scientific world was ill prepared for Koch's revolutionary statement in 1901 at the British Congress on Tuberculosis in London to the effect that "human tuberculosis differs from bovine tuberculosis and cannot be transmitted to cattle", and also that "bovine tuberculosis is so rarely transmitted to man that it can safely be ignored as a source of tuberculosis, and no special protective measures are indicated."

The first part of this statement - that human tuberculosis differs from bovine tuberculosis (a reversal of his former opinion) is essentially correct, and has been fully confirmed. In the second part of his statement it will, however, be observed that Koch went much further than Smith and his followers in America who, while carefully distinguishing between the human and bovine types, never denied the pathogenicity of the latter for humans. It is not surprising that Koch went astray when we consider the relative rarity of primary abdominal tuberculosis in Germany as compared with the position in England, and indeed Koch himself in his address, gave post mortem statistics derived from various German sources, in support of his claims. (At a later date Gohn and Kudlich, (1930) in a study of 2114 cases of childhood tuberculosis in Central Europe, found a primary intestinal infection in only 24 (1.14%).

Although Koch abandoned this extreme view later (Lange, 1932), it would be difficult to exaggerate the immediate effect of Koch's announcement. The prominence of the man, and the startling nature of his conclusions, aroused tremendous interest in the subject. It at once completely paralysed all the many efforts which were then being made in the interests of public health, to stamp out

tuberculosis/....

tuberculosis amongst herds of cattle, and on the other hand it is probable that no single statement has ever done so much to stimulate experimental investigation: Great Britain appointed a Royal Commission which carried out a long series of experiments without interruption for nine years: Germany also appointed a commission, and important researches were carried out in France, Denmark, Holland, Sweden and Japan, while in America, Smith continued with his researches (Smith, 1904-5 and Smith and Brown, 1907). Working in the research laboratories of the New York Department of Health, Park and Krumweide (1910) produced a monumental work on human and bovine tubercle bacilli, and this, in conjunction with the final report of the British Royal Commission (1911), finally served to dispel all doubt concerning the pathogenicity of the bovine tubercle bacillus for human beings.

From the pathological point of view chief interest still centres round the pathogenesis of the disease.

Rich (1929) and Rich and McCordock (1929; 1933), introduced a different (if not new) concept by maintaining that the cause of diffuse tuberculous meningitis could invariably be traced to some contiguous tuberculous focus discharging its contents into the subarachnoid space. To substantiate this belief they quoted the results of an examination of a series of 82 cases in which they claimed to have found such foci in 77, i.e. 94% of cases.

Prior to this, and despite evidence by Kment (1924), Korteweg (1926) and others to the contrary, the general belief had been that sufficient tubercle bacilli could escape from the blood stream into the meninges to immediately produce a diffuse tuberculous meningitis.

A great deal of evidence has accumulated against this theory of a direct haematogenous pathogenesis of the disease, but positive proof of the only reasonable alternative/.....

alternative (Rich's theory), by the actual demonstration of contiguous foci, has been more difficult to come by.

As a result of their investigations, Radmann (1935), MacGregor and Green (1937), McMurray (1944), Schwarz (1948) agree with Rich, but Ragins (1936) and Beres and Meltzer (1938) frankly disagree and Beitzke's (1939) conclusions are cautious and reserved.

However, with the sole exception of MacGregor and Green, conclusions are based on small series of cases, and the third object of this investigation then is to test Rich's theory by studying a numerically comparable series of cases.

SOURCE AND NATURE OF BACTERIOLOGICAL MATERIAL

This investigation was commenced in June, 1950, and all the bacteriological material had been collected by July, 1951.

Conclusions are based on results obtained from cultures of organisms derived from 200 individual cases of tuberculous meningitis, 163 cases of which originated in the Cape Peninsula, and 37 cases in various towns of the Western Province of the Cape and beyond, as set out in the maps of these areas. Figure I.

The racial distribution of the cases was as follows:-

Cape Coloureds	164 cases
Natives	17 "
Europeans	16 "
Asiatics	3 "

The preponderance of Cape Coloureds in this series is very striking, and can be correlated with the social and economic conditions under which they exist.

These conditions may be assessed from the following paragraph quoted verbatim from the latest (June, 1949) Annual Report of the Medical Officer of Health, Cape Town:

"The social and economic conditions of the Cape Coloureds are on the whole unsatisfactory. A part of them have skilled trades and earn good wages but the majority are unskilled labourers and many of the men earn less than 70 shillings a week when in full work. The position is aggravated by the large size of the families, but the family income is eked out when possible by earnings brought in by the wife and children. The measures taken for the relief of distress are inadequate, and there is no compulsory insurance against sickness. There is much undernourishment and housing accommodation is expensive and bad. The social and cultural level is low. The principle of compulsory education does not apply to non-Europeans, and, though there are some good

Coloured/.....

Please Turn Over.

TABLE I.

RACIAL AND AGE INCIDENCE OF BACTERIOLOGICAL MATERIAL.

Race.	AGE IN YEARS.																					
	Total.	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	20-30	30-40	Above 40
Europeans.	16	6	2	-	1	1	1	-	-	-	-	1	-	1	-	1	-	-	1	-	1	-
Indians.	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
Natives	17	4	4	1	1	1	3	-	-	1	-	-	-	-	-	-	-	-	-	2	-	-
Coloureds.	164	38	46	20	11	8	10	4	4	1	3	1	1	5	1	-	2	1	2	5	1	-
Chinese.	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Total.	200	48	52	21	13	10	14	4	4	2	4	2	1	7	1	1	2	1	3	7	2	1
% of Total.		24	26	10.5	6.5	5	7	2	2	1	2	1	.5	3.5	.5	.5	1	.5	1.5	3.5	1	.5

Coloured schools, the general level of schooling is low, and there is a lack of discipline in adolescents and a serious problem caused by Coloured delinquency. The illegitimacy rate is high and venereal disease is rife. The social contrast between the Europeans and Cape Coloureds can be expressed by the statement that whereas in the Whites it is only a small minority that belong to the depressed classes, in the Cape Coloured it is the majority. The same contrast is seen in the housing conditions; it is a small minority of Europeans who live in slum conditions, but a majority of the Coloured".

The non-European cases from towns in the Western Province were all children of Coloured labourers amongst whom even worse conditions prevail, as organised relief work is inadequate, and liquor (especially amongst farm labourers) is more readily obtainable.

Eighty-nine (44.5%) of these 200 cases were males. Schwarz (1948) in a review of 22,489 cases of tuberculous meningitis described in the literature, found males to constitute 51.5 per cent of cases, and although this is the usual finding, a preponderance of females is not unknown as Kinnear (1925) reported an incidence of 44% males and Harrington (1939) in this country also recorded an incidence of 43.6% males, in series of 100 and 78 cases respectively.

The age distribution of the cases follows the pattern of surveys made in many countries, and the tendency for tuberculous meningitis to develop in the early years of life is well brought out in this series where 50% of cases occurred during the first two years of life, as depicted in Table I on opposite page.

The fact that no case occurred below the age of three months/.....

months (the youngest case was 4 months old) is in conformity with the findings of Siegel (1935) who, in an extensive review of the literature, found a few cases occurring during the third month of life but no undoubted cases in infants of two months or younger.

Working in a Public Health Laboratory, my position has been particularly favourable for an investigation of this nature, as specimens from practically all cases to be investigated in this area reach this laboratory either directly from the doctor in charge of the patient, or via the various hospitals once the patient has been hospitalised.

Hospitals drained were the following: City Hospital for Infectious Diseases, Stellenbosch Sanatorium, Brooklyn Chest Hospital, Langa Hospital, Caledon Hospital and through the courtesy of various doctors also Groote Schuur Hospital and the New Somerset Hospital, the last two normally admitting few cases of tuberculous meningitis.

Of these 200 cases, 196 were obtained from the above mentioned hospitals as follows:-

City Hospital for Infectious Diseases	173 cases
Brooklyn Chest Hospital	10 cases
Stellenbosch Sanatorium	5 cases
New Somerset Hospital	3 cases
Groote Schuur Hospital	3 cases
Langa Hospital	1 case
Caledon Hospital	1 case

The remaining four cases (three from Somerset West and one from Piketberg) were not hospitalised.

It is thus possible to state that apart from few exceptions, practically every case diagnosed, and many cases suspected of having tuberculous meningitis, occurring in the Western Province during the above mentioned period of collection, were investigated.

During life, the material investigated was cerebrospinal fluid obtained under strictly sterile conditions, either by the lumbar route, by cisternal puncture or from the ventricles of the brain in a few cases.

With/.....

With cases dying before fluid could be obtained (6 cases), or before ante-mortem cultures were known to be positive (31 cases), post-mortem isolation was attempted. This material consisted of cisternal or ventricular fluid taken under sterile conditions, and exudate obtained either by swabbing the base of the brain with an ordinary throat swab, or simply by cutting a portion of it away.

In 18 cases caseous lymph nodes were also cultured.

Fluid was collected in 5" x $\frac{1}{2}$ " hard glass test tubes which were fitted with cork stoppers and had previously been autoclaved at 15 pounds pressure for 30 minutes.

These tubes were used because they fitted the particular centrifuge, so that once the fluid had been collected, it could be centrifuged without any further handling. A large volume of fluid was usually dealt with, for, the cerebro-spinal fluid being under increased pressure, it was often possible to collect 10 cc. in each of four separate test tubes. An attempt was made to collect an equal volume in each tube. This procedure was considered preferable to the use of fewer test tubes containing more fluid, in the event of possible contamination of one of the tubes.

To ensure minimal delay in getting specimens to the laboratory and for various other reasons, all specimens were obtained personally from the various hospitals, or from the homes of the patients not hospitalised, as was the case with the three patients from Somerset West, and one patient from Picketberg.

The material examined in the course of this investigation is set out in Table II.

Table II/.....

TABLE II
MATERIAL EXAMINED

Nature of Specimen	Ante-Mortem or Post-Mortem	Number of Cases	Number of Specimens
C.S.F. from Suspected Cases	Ante-Mortem	200	768
Tuberculous C.S.F.	Post-Mortem	37	74
Basal Tuberculous Exudate	Post-Mortem	37	111
Caeceus Abdominal Lymph Nodes	Post-Mortem	13	13

Total Number of Specimens Investigated:- 971

ISOLATION OF ORGANISMS

Two methods are available for the isolation of tubercle bacilli. Firstly, by direct culture of the tuberculous material on suitable media and secondly, by the inoculation of the tuberculous material into a susceptible animal and making cultures from the lesions eventually resulting. The laboratory animal universally used is the guinea pig.

Although the first method (that of direct culturing) has been in use since Koch (1882) described the first suitable medium (inspissated bovine or sheep serum) it was not until the last two decades that it has gradually come to be regarded by most workers as at least as effective a method as the animal inoculation procedure.

Both methods have disadvantages. If sole reliance for isolation is placed on the guinea pig, then at least two animals must be inoculated, in case one of the animals should die of intercurrent disease before the injected tubercle bacilli have caused manifest disease in the animal. Besides being costly, accommodation facilities must be doubled. Also as Blacklock (1932a) points out, the resistance of individual guinea pigs to tuberculous infection varies, and small inoculated doses may on occasions fail to cause disease in a particular animal. A further possible disadvantage to the sole use of guinea pigs is the fact that in small doses they are relatively resistant to avian infections, and although the incidence of human avian infection is completely insignificant, it is as well to allow for such an occurrence in an investigation of this nature.

As regards isolation by means of direct culture, small numbers of these strictly parasitic organisms transferred from animal tissues, may not be able to adapt themselves in time to the artificial conditions of even the

most/....

most satisfactory culture medium, and such a strain will then be lost if sole reliance is placed on this method. This is a well known danger particularly with regard to the isolation of the bovine bacillus (Holm and Lester, 1941), and it was therefore decided to employ both methods of isolation in this investigation.

Throughout this investigation all inoculations of media, post mortem examinations on guinea pigs whose tissues were intended for subsequent culture and the preparation of suspensions of tubercle bacilli for rabbit inoculation, were done in an inoculation box of the type described by Mackie and McCartney (1948). The floor of the box was covered with a towel soaked in a 1:1000 solution of mercuric bichloride, and in addition to the relative absence of dust and currents of air, it afforded some protection to the worker.

At frequent intervals it was disinfected by vaporising formalin in it.

The procedure with cerebro-spinal fluid taken ante- or post-mortem was as follows:

As the fluid was obtained under sterile conditions and transported personally to the laboratory, treatment with acids or alkalis to destroy contaminants was not considered necessary, and accordingly the test tubes containing the fluid were simply centrifuged at a rate of 2500 r.p.m. for one hour.

The test tubes were then transferred to the inoculation box and all but the last 2-3 cc. of fluid removed from each by means of sterile pasteur pipettes. Keeping the contents of each tube separate, half the tubes (one or two) were then used for seeding the different media employed, while the other half was used for guinea pig inoculation. In the event of there being an uneven number of tubes (a rare occurrence), the odd tube was also seeded/.....

seeded onto media. The contents of each tube used for seeding was divided as evenly as possible (by means of drops) between the different media, and distributed over the surface of these media by means of pasteur pipettes. The fibrinous clot, when present, was implanted on the surface of a medium. In the event of there being two tubes for inoculating onto media, only half the media, i.e. one set of four, was inoculated with the one tube, and half with the contents of the other tube, to avoid the possibility of all cultures being contaminated as the result of other organisms being present in the contents of one of the tubes.

The volume of fluid dropped onto each medium usually amounted to about 0.5 to 1.0 cc. This relatively large volume of fluid tended to combat drying of the medium resulting from 2-4 weeks' incubation.

The screw cap stoppers of the culture bottles were then tightened, and the bottles placed in a media sloping tray and incubated at 37 degrees Centigrade for two weeks. If no growth was present at this stage the bottles were returned to the incubator for another two weeks before a final decision as to the presence or absence of growth was recorded.

Because of the limited accommodation available only one guinea pig was used for each case, the inoculum invariably being cerebro-spinal fluid obtained ante- or post mortem. No other material was ever used for guinea pig inoculation. The animal was injected intramuscularly in one or other, or both thighs with the remaining half the volume of fluid. A wide bore needle was used to enable the fibrinous clot present in many of the specimens also to be deposited in the tissues of the animal.

After a period of six to ten weeks, surviving guinea pigs were killed and examined for tuberculous lesions, and
although/...

although cultures were not invariably made from these lesions, the macroscopic diagnosis was invariably confirmed by microscopical examination of exudate or sections.

By starting off with roughly equal volumes of fluid in even numbers of test tubes, and halving the tubes between animal inoculation and cultural methods of isolation, a minimal sampling error was introduced and comparisons of results of the two methods could be made. Also, as the volume of fluid for the direct cultural method was fairly evenly divided between the various media employed, deductions could be drawn as to the most satisfactory medium used in this investigation.

Where cultures were made from tuberculous guinea pigs, the procedure was as follows: Working in the inoculating box, the enlarged and caseous inguinal, iliac and lumbar glands as well as the spleen and liver were dissected and removed intact to sterile petri dishes after gently searing their surfaces in a bunsen burner. In cases where the inguinal glands were ulcerated and thus almost certainly contaminated, these glands were discarded.

The capsules of the glands were then incised with a sterile scalpel and their contents rubbed over the surfaces of the media by means of a wire loop. The liver and spleen were similarly cut up and pieces of the organs rubbed over the surfaces of the media employed. After the addition of about 1 cc. of sterile saline to the bottles, the latter were stoppered and incubated upright at 37 degrees Centigrade for periods varying between 3 and 6 weeks.

Autopsy material likely to be contaminated with other organisms was, as has been mentioned in the previous chapter, obtained either by swabbing the base of the

brain/.....

brain with an ordinary throat swab, or simply by cutting away a portion of the exudate at the base of the brain. In 18 cases caseous mesenteric lymph nodes were also cultured.

Homogenisation of the material and simultaneous destruction of contaminating organisms was attempted by the trisodium phosphate method of Corper and Stoner (1946) and the acid peroxide iron method of Jungmann (1938), the material being equally divided. Jungmann's method was originally devised for liquifying sputa - the sulphuric acid and the hydrogen peroxide having a marked solvent action on mucus in the presence of traces of iron (Nassau, 1942). Concentration of the tubercle bacilli in the homogenised material was achieved by centrifuging the latter.

The swabs were treated as follows: One swab (invariably two were available) was immersed in 10 cc. of 10% solution of trisodium phosphate (23% solution of the hydrated salt) contained in a hard glass test tube. This tube was subjected to repeated shaking over a period of 24 hours. In the intervals between shaking, the test tube was kept at 37 degrees Centigrade in an incubator. After 24 hours had elapsed the swab was removed and the tube centrifuged at a 2500 r.p.m. for 60 minutes. Working in the inoculating box, all but the last 1 cc. of fluid was pipetted off and the alkali neutralised by filling the tube with sterile saline. The tube was then again centrifuged for 60 minutes and the last 1 cc. seeded onto media in exactly the same way as the cerebro-spinal fluids were dealt with.

The other swab was immersed in 10 cc. of Jungmann's acid peroxide iron mixture. This mixture consists of equal volumes of two solutions "A" and "B". Solution "A" consists of 20 gm. of ferrous sulphate, 20 cc. of concentrated sulphuric acid and 180 cc. of distilled water.

Solution "B" consists of 1 volume per cent of hydrogen/....

hydrogen peroxide made up freshly each time by diluting 5 cc. of 20 volumes per cent hydrogen peroxide to 100 cc. with water.

This swab was dealt with in exactly the same way as the previous one apart from the important difference that it was removed after 10 minutes (instead of 24 hours) and centrifuging commenced immediately.

Lymph nodes and exudate were dealt with by cutting them up (if necessary) into small pieces and grinding the material in an agate mortar until it was homogenous. The material was then halved and the halves treated with the trisodium phosphate and acid peroxide iron methods respectively in exactly the same manner as described above.

The four landmarks in the history of the cultural examination of the tubercle bacillus are the following: Firstly Koch (1882) managed to cultivate the bacillus on inspissated serum; this was followed by Nocard and Roux (1887) who noted the marked stimulating effect glycerol has on the bacillus. They accidentally discovered this by adding glycerol (a hygroscopic substance) to serum media in an effort to prevent the media from drying out on incubation.

Almost immediately afterwards Pawlowsky (1888) devised a potato cylinder medium which sustained the growth of the tubercle bacillus, and finally Dorset (1902; 1903) described an inspissated whole egg medium which also promoted the growth of the bacillus.

Since then, nearly every bacteriologist interested in the subject of tuberculosis has deemed it necessary to introduce a "new" medium (Fiassecka-Zeyland, 1939). This medium, which is usually only a modification of some existing medium, bears the name of its originator and usually receives its greatest approbation from the same source.

Thus/.....

Thus practically all of the media use all, or some combination of, the fundamental ingredients, protein, carbohydrate, fat and minerals in the form of eggs, milk, cream, potatoes, glycerol and serum or amino acids. More recently additional mineral salts have been introduced as nutrients and buffering agents, and dyes such as gentian (or crystal) violet, malachite green or congo red have been added in minute amounts to act as bacteriostatic agents against contaminants.

Apart from the investigations by Herrold (1931), Corper and Cohn (1933), Schwabacher (1936-37), Dubos and Davis (1946), Dubos and Middlebrook (1947) and Corper and Chan (1952), few attempts have been made at defining the exact nutrient requirements of the tubercle bacillus and I agree with the opinion of Corper and Cohn (1942) that most media are simply compounds of two or more of the ingredients mentioned above, reminiscent of the "shot gun" prescriptions in therapeutics of bygone days.

Thus, blood, potatoes and glycerol have been combined with agar in various proportions to give modifications of the Bordet-Gengou medium by Mishulow (1932), Alexander (1934) and Tarsnis and Frisch (1951).

Pawlowsky's original potato cylinder has been modified by Corper and Uyei (1928; 1929; 1930) by the addition of crystal violet to the potato.

Dorset's original egg medium was modified by Lubenhau (1907) who replaced the water in the original medium with glycerine broth, by Petroff (1915) who added Gentian Violet to the medium and by Wooly and Petrik (1931) who replaced the glycerine broth by a glycerinated potato extract and changed the dye to crystal violet.

The use of whole egg has been questioned by workers like Herrold (1931), Corper and Cohn (1933), Blacklock

and/.....

and Griffin (1935) and Schwabacher (1936-37) who have suggested the use of 2-3 volume egg yolk to one volume glycerinated potato extract, one volume of glycerinated water or saline respectively. Herrold uses nutrient agar to overcome the process of inspissation.

More complicated media have been suggested by Petraghiani (1926) and Sweany and Evanoff (1928) consisting of milk, cream, peptones or meat extracts, potatoes and glycerol. Lowenstein (1924; 1933) compounded whole eggs, potato flour, glycerol and a dye (malachite green) and added various mineral salts as additional nutrients and buffering agents. This medium has subsequently been modified in the amount of the different nutrients added and the nature of the buffering agents employed, by Jensen (1932) and Holm and Lester (1941).

Purely synthetic media have been introduced by Proskauer and Beck (1894), Long (1919), Long and Siebert (1926) and Kirchner (1932). These media consist of amino acids (usually asparagine) and various mineral salts functioning as additional nutrients and buffering agents. The above media have been modified by numerous workers, usually by the addition of serum or further protein (Dubos and Middlebrook, 1947; Lowry and Berry, 1950). Growth on these fluid media usually appeared as a surface pellicle and Dubos (1945) obtained diffuse subsurface growth in Long's synthetic medium by adding to it a dispersing agent in the form of an Oleic acid ester (Tween 80). On hydrolysis, however, free oleic acid is liberated which is toxic to the tubercle bacillus. This toxicity is overcome by the addition of an albumin fraction of bovine serum to the medium.

The media used for isolation in this investigation were the following:-

(1) Three/...

- (1) Three parts whole egg (by volume) to one part water (Dorset, 1902).
- (2) Three parts egg yolk (by weight) to one part water with glycerol added to make a 3% solution (Corper, 1933)
- (3) Three parts egg yolk (by volume) to one part potato extract (modified Blacklock and Griffin, 1935).
- (4) A buffered whole egg-potato flower medium (Lowenstein as modified by Jensen, 1932).

The Blacklock and Griffin medium was modified simply by the omission of glycerol.

It will be observed that two of these media contain no glycerol. This is very important (as will be discussed later), for isolation on glycerine free media is essential if reliance is to be placed on various cultural characteristics of the organisms for type determination.

Crystal violet was added to the first three of these media in very low concentrations not for any bacteriostatic action which it may possibly exert on the growth of contaminants, but simply to give a slight colour contrast to the media.

The Lowenstein Jensen medium was not modified in any way.

Fresh media were prepared at weekly intervals, and dispensed in roughly 5 cc. amounts in sterile 1 oz. McCartney bottles which were then sloped in an inspissator at 80-85 degrees Centigrade for exactly 2½ hours. After 24 hours incubation, the sterile media were ready for use.

The concentration of the crystal violet added was reduced from the usually recommended bacteriostatic concentration of 0.01% to 0.0025% (0.5 cc. of a 0.5% watery solution of crystal violet to every 100 cc. of medium).

The malachite green dye was added in the bacteriostatic dose recommended - 0.025%.

Throughout the investigation new laid eggs were

obtained/.....

obtained from the same source, and were soaked in 70% ethyl alcohol for 15 minutes before use. They were then dried and broken into sterile measuring cylinders after separation of the yolk if necessary. This egg fluid was homogenised in a Waring blender, and was then ready for incorporation in the various media.

Dorset's medium was compounded by adding one part (by volume) of sterile water to three parts of whole egg fluid and dispensing as described above after the addition of 0.0025% of crystal violet.

Corper's medium was prepared by the addition of three parts (by weight) of sterile egg yolk to one part of sterile water, and adding 3% glycerol to the mixture. By performing a simple experiment, the weight measurements were converted to volumetric ones, thus facilitating the making of the medium. After the addition of the crystal violet it was dispensed as above.

The potato extract of Blacklock and Griffin's medium was prepared by boiling one part (by weight) of very thinly sliced potatoes in four parts of water for half an hour, and then letting the sediment settle. The supernatant was the potato extract. This was added to egg yolk fluid in the proportion (by volume) of one part potato extract to three parts egg yolk. After adding 0.0025% of crystal violet, it was ready for dispensing.

Lowenstein Jensen medium was prepared as follows:- Using only analytic grade reagents, the mineral buffering solution consisted of 0.4% potassium dihydrogen phosphate, 0.04% magnesium sulphate, 0.4% magnesium citrate, 0.6% asparagin and 2% glycerol in distilled water. The solution was placed in an Arnold steamer for two hours and then allowed to cool overnight.

The/.....

The next day 5% potato starch was added to the solution in a boiling water bath until a satisfactory paste was produced, and then allowed to remain in a water bath at 56 degrees Centigrade for an hour. Whole egg fluid was then added in the proportion of 5 parts (by volume) egg fluid to three parts salt starch solution.

After the addition of 0.025% malachite green, the medium was dispensed and inspissated as described above.

Results.

The results of guinea pig inoculation are the following:

Ante mortem cases were derived from cerebro-spinal fluid obtained from 200 cases of suspected tuberculous meningitis. Of these, four cases subsequently proved to be non-tuberculous (three *H. influenzae* and one treated case of pneumococcal meningitis). Disregarding these four cases, the results can be tabulated as follows:

Table III.

TABLE III.

ANTE-MORTEM ISOLATION BY GUINEA-PIG INOCULATION.

Number of Guinea-Pigs inoculated	Number of Guinea-Pigs who died prematurely of inter-current disease.	No evidence of Tuberculosis after 6-10 weeks	Tuberculosis Positive after 6-8 weeks.	% Positives.
196	10	4	182	91.8

Post mortem cases were derived from 6 cases not obtained during life and another 31 cases who died before results of their ante mortem cultures were known to be positive.

The results of these 37 cases are set out in Table IV.

Table IV/.....

TABLE IV
POST-MORTEM ISOLATION BY GUINEA-PIG INOCULATION

Number of Guinea-Pigs Inoculated	Number of Guinea-Pigs who died prematurely of Intercurrent disease	No evidence of Tuberculosis after 6-10 weeks	Tuberculosis Positive after 6-8 weeks	% Positives
37	6	0	31	83.7

Ante mortem and post mortem results can be combined as follows: Table V.

TABLE V.
COMBINED RESULTS OF GUINEA-PIG INOCULATION

Number of Individual cases.	Number of Guinea-Pigs Inoculated.	Number of POSITIVE Guinea-Pigs	Number of NEGATIVE Guinea-Pigs	Number of Guinea-Pigs who died of Intercurrent Disease.	Number of POSITIVE cases.	% POSITIVE cases.
196+6	233	213	4	16	199	93.0.

In 36 cases it was necessary to obtain cultures from tuberculous guinea pigs. The material was only planted onto Dorset's medium, and in every case all the cultures inoculated were positive - there being no failures and no contamination. The impression is that material from lymph nodes yielded the most abundant growth.

Results of direct cultural isolation are the following: Of the 200 cases from which cerebro-spinal fluid was obtained during life, four, as mentioned above, were based on wrong diagnoses, leaving 196 individual cases to be considered.

These results are set out in Table VI.

TABLE IX

RESULTS OF GUINEA-PIG AND CULTURAL TESTS.

No. of Individual Cases Investigated.	POSITIVE by Guinea-pig Inoculation.		POSITIVE by Culture		POSITIVE G-pig NEGATIVE Culture		NEGATIVE G-pig POSITIVE Culture		NEGATIVE G-pig and Culture	
	No of Cases	% Positive	No of Cases	% Positive	No of Cases	% Positive	No of Cases	%	No of Cases	%
202	188	93.0	197	97.5	3	1.4	12	5.4	2	.9

TABLE VI

ANTE-MORTEM ISOLATION BY CULTURAL METHODS.		
No. of Cases.	No. of Cases isolated by Culture.	% Cases Isolated.
196	191	97.3

Post mortem cerebro-spinal fluid was obtained from six cases not obtained ante mortem and 31 cases where death occurred before the ante mortem cultures were known to be positive.

These results are set out in Table VII.

TABLE VII

POST-MORTEM ISOLATION BY CULTURAL METHODS.		
No. of Cases.	No. of Cases isolated by Culture.	% Cases Isolated.
37	34	91.8

These results can be combined as follows: Table VIII.

TABLE VIII

COMBINED RESULTS OF CULTURAL METHODS.		
No. of Individual Cases.	No. of Cases Isolated.	% Cases Isolated.
196+6	197	97.5

In Table IX on the opposite page the results of guinea pig and cultural tests are compared.

In comparing the results of guinea pig inoculation and direct culture, the following must be kept in mind. Firstly, the results must be strictly comparable by making the quantities of material seeded on media and inoculated into guinea pigs equal, thus introducing the smallest possible sampling error (Berg, 1941).

Secondly, the type of tubercle bacilli found in the specimens must be considered, for although my bovine

strains/.....

strains were positive by both methods, it is an established fact (Holm and Lester, 1941; McIntosh et al., 1945) that guinea pig inoculation is superior to direct cultivation for isolation of the bovine bacillus, especially if glycerine containing media are used (Evanoff and Swesny, 1929; Karlson, 1940).

Thirdly the nature and number of media utilised, as also the number and state of health of the guinea pigs used, will affect the results, for as Cruickshank (1951) has shown it is necessary to inoculate 3 tubes of media to obtain the maximum number of positive cultures. If only one tube is used per case, the cultural method is not functioning as efficiently as possible, for it sometimes happens that, due to scanty numbers of organisms being present in the inoculum or due to faulty technique, with two or more tubes of the same medium inoculated with the same material, one or more of the tubes may show no growth or be overgrown with contaminants, in the presence of other tubes showing colonies of tubercle bacilli.

Also guinea pigs may die of intercurrent disease, and in the event of only one guinea pig being used per case, the result is immediately negative as far as guinea pig isolation is concerned.

This latter consideration largely explains the disparity (not really statistically significant as $P = 0.014$) existing between results of the two methods of isolation for although the same total quantity of fluid was used for the guinea pig and cultural methods, the fluid for the latter method was distributed over at least four (usually eight) media, while sole reliance was placed on only one guinea pig. An analysis of uncompleted cases is set out in Table X.

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TABLE X.

Number of Cases.	ANALYSIS OF UNCOMPLETED CASES.			
	Uncompleted Tests.			
	Guinea-Pigs.		Culture Sets.	
	Number	Percentage	Number	Percentage
196 (Anti-Mortem)	10	5.1	5	2.5.
37 (Post-Mortem)	6	16.2	3	8.1.

Uncompleted tests refer to procedures which had to be abandoned because of the death of the guinea-pig due to intercurrent disease, or contamination of all tubes of a culture set.

There have been numerous reports of comparisons of the two methods of isolation in the literature, some authors not paying enough attention to the above mentioned points, but suffice it to say that practically every known medium has at one or other stage of its career been pitted against the guinea pig with various and variable results. However, workers like Stadnischenko and Sweany (1931), Feldman (1931), Herman et al. (1932), Guggenheim and Finkelstein (1939), Hoyt et al. (1939-40), Whitehead (1940) and Sasona et al. (1941) have reported better results with guinea pig inoculation while Herrold (1931), McNabb and Wilson (1932), Norton et al. (1932), Hirschberg (1933-34), McNabb (1936), Ukil and Thakurta (1936), Murphy and Duerschner (1938-39), Holm and Lester (1941), Roberts et al. (1950) and Weed and Needham (1950) have had greater success with cultural methods of isolation.

In every reported series, however (as also in my series), cultures are positive for a particular case when the corresponding guinea pig is negative and visa versa, and despite the advantage which the guinea pig offers of automatically distinguishing between saprophytic and pathogenic mycobacteria (Weed, 1951) the logical conclusion (which most of the above authors advise) is to employ both methods of isolation for each specimen (Friedmann and Rodan, 1947).

Thus/.....

TABLE XI.

RESULTS OF CULTURAL EXAMINATION OF CEREBRO-SPINAL FLUID.

Type of Specimen	Number of cases.	DOBERT'S								BLACKLOCK'S								CORPER'S								LOWENSTEIN'S							
		No of tubes	POS	%	NEG	%	Con-tam.	%	No of tubes	POS	%	NEG	%	Con-tam.	%	No of tubes	POS	%	NEG	%	Con-tam.	%	No of tubes	POS	%	NEG	%	Con-tam.	%				
ANTE-MORTEM C.S.F.	186 (2 tubes from 180 cases 1 tube from 16 cases.)	376	346	92	25	6.6	5	1.4	376	323	85.9	28	10.1	15	4	376	308	82	57	16.1	11	2.9	376	331	88	36	9.6	9	2.4				
POST-MORTEM C.S.F.	37 (2 tubes from each case)	74	66	89.7	2	2.7	6	8.1	74	40	54	28	37.9	6	8.1	74	57	77	11	14.9	6	8.1	74	54	73	14	18.9	6	8.1				

Thus by employing both methods of isolation in this investigation, 200 cases were isolated out of a total of 206 attempted cases. Four of the six failures can be attributed to misdiagnoses (3 *M. influenzae meningitidis* and one case of treated pneumococcal meningitis) leaving two cases to be accounted for.

These two cases were negative by both methods (uncompleted in both instances), but acid and alcohol fast bacilli morphologically resembling *M. tuberculosis* were eventually found on direct microscopy of the cerebro-spinal fluid and the subsequent clinical course of these two patients tended to confirm the diagnosis of tuberculous meningitis which was thus missed by both methods of isolation. This is not a very uncommon occurrence and in a series of 18 cases of tuberculous meningitis, Smith, Vollum and Cairns (1948) also describe one case where both cultural methods were negative, despite demonstrating the presence of the organisms in direct smears.

As the fluid was equally divided between the various media used, comparisons as to the efficiency of the media employed can be made. This is set out on the opposite page in Table XI.

Combining ante mortem and post mortem results, the following figures are obtained: Table XII.

TABLE NO. XII.

COMBINED ANTE-MORTEM AND POST-MORTEM RESULTS.

	Total		Dorset's		Blacklocks		Corper's		Lowenstein's.	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total Specimens	450	100	450	100	450	100	450	100	450	100
Positive Specimens	439	97.6	412	91.6	363	80.7	365	81.1	365	85.6
Negative Specimens	0									
Tests Contaminated	11	2.4								
Number of tubes Contaminated	64	14.2	11	2.4	21	4.7	17	3.8	15	3.3

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Negative Specimens	0									
Tests Contaminated	11	2.4								
Number of tubes Contaminated	64	14.2	11	2.4	21	4.7	17	3.8	15	3.3.

These results indicate a statistically significant difference in the efficiency of Dorset's medium over all the other media - Dorset's versus Blacklock's, $P < 0.00005$; Dorset's versus Corper's, $P < 0.00005$; and Dorset's versus Lowenstein's, $P = 0.0023$ - and tend to confirm the findings of Griffith and Griffith (1907), Herrold (1931), Corper and Cohn (1933), Schwabacher (1937) and McCarter and Kanne (1942) that a medium consisting of 2-3 parts whole egg or egg yolk to one part water or saline, is as, or more, efficient than other more complicated media, i.e. the Lowenstein-Jensen medium.

The fact that a glycerine free medium (Dorset) was the most successful, and that by the use of two glycerine free media (Dorset's and Blacklock's) all but one of the isolated strains was obtained on one or other or both of these media, tends to confirm the findings of Corper and Cohn (1933) and Dubos and Middlebrook (1947) that despite the marked effect which glycerol has on the growth of certain strains of tubercle bacilli, it is not essential for the initiation of growth on artificial media.

In the one instance where both these media were negative, growth was obtained on a glycerol free medium by culturing the tissues of the corresponding guinea pig.

Replacement of the water in the Dorset type of medium by a potato extract (done in the potato egg medium of Blacklock and Griffin) serves no useful purpose as can be seen from the relative inefficiency of this medium, and has been remarked upon by Corper and Cohn (1933).

As each set of four media were invariably inoculated from the contents of one test tube, the slight disparity in the contamination rates of the different media must be ascribed to faulty technique, and it is worthy of note that Lowenstein's medium with its dye (malachite green) in supposedly bacteriostatic concentrations

(0.025%)/.....

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(0.025%)/.....

TABLE XIII.

RESULTS OF CULTURAL EXAMINATION OF BASAL EXUDATE AND GLANDS.

TRISODIUM PHOSPHATE METHOD.

Type of Specimen	Number of cases.	DORSET'S								BLACKLOCK'S								DORPER'S								LOWENSTEIN.							
		No of tubes	POS	%	NEG	%	Con-tam.	%	No of tubes	POS	%	NEG	%	Con-tam.	%	No of tubes	POS	%	NEG	%	Con-tam.	%	No of tubes	POS	%	NEG	%	Con-tam.	%				
Exudate from Base of Brain	37 (Swab & 1 Portion of Exudate.	74	71	96	0	0	3	4	74	62	83.8	9	12.2	3	4	74	69	79.8	12	16.2	3	4	74	71	96	0	0	3	4				
Mesenteric Glands	18	18	18	100	0	0	0	0	18	18	100	0	0	0	0	18	18	100	0	0	0	0	18	18	100	0	0	0	0				

ACID PEROXIDE IRON METHOD.

Type of Specimen	Number of cases.	DORSET'S								BLACKLOCK'S								DORPER'S								LOWENSTEIN.							
		No of tubes	POS	%	NEG	%	Con-tam.	%	No of tubes	POS	%	NEG	%	Con-tam.	%	No of tubes	POS	%	NEG	%	Con-tam.	%	No of tubes	POS	%	NEG	%	Con-tam.	%				
Exudate from Base of brain	37 (Swab & 1 Portion of Exudate.	74	67	90.5	0	0	7	9.5	74	62	83.8	0	0	12	16.2	74	53	71.6	9	12.2	12	16.2	74	71	96	1	1.3	2	2.7				
Mesenteric Glands	18	18	15	83.3	0	0	3	16.7	18	16	88.9	0	0	2	11.1	18	15	83.3	0	0	3	16.7	18	16	88.9	0	0	2	11.1				

(0.025%) had a slightly higher contamination rate (not significant however) than Dorset's medium to which very low concentrations (0.0025%) of crystal violet had been added merely to obtain a slight colour contrast between the medium and the colonies of tubercle bacilli, and tends to support the findings of Corper and Cohn (1933).

While no attention was paid to the rate or quantity of growth on the different media, the impression is that colonies tended to appear earlier and be more numerous as a rule on Lowenstein's medium, and an interesting fact is that quite regardless of the medium in which the fibrinous clot was imbedded, growth invariably occurred around the clot provided there was no contamination present.

As regards the results of culture of contaminated material treated with the concentrating agents, cultures were obtained from each case by both methods employed and although no really significant difference existed in the total positive rates of the two methods ($P=0.0107$), the significant difference present in the total contamination rates ($P<0.00003$) proved the trisodium phosphate procedure to homogenise material and destroy contaminating organisms more satisfactorily than Jungmann's method as shown on the opposite page in Table XIII.

Combining the results of the two methods of concentration in Table XIV (below) it is seen that Lowenstein's

TABLE NO. XIV.

RESULTS OF COMBINED CONCENTRATION METHODS.

	Total		Dorset's		Blacklocks		Corper's		Lowenstein's.	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total Specimens	184	100	184	100	184	100	184	100	184	100
Positive Specimens	184		171	92.9	158	85.9	145	78.8	170	92.7
Negative Specimens	0									
Tests Contaminated	0									
Number of tubes Contaminated	53	29.9	16	7.1	17	9.2	18	9.8	7	3.8

medium/.....

medium replaces Dorset's medium as being most satisfactory although the disparity is not statistically significant ($P = 0.13$). While the difference existing between the results obtained on Dorset's and Corper's media are again significant ($P < 0.00005$), comparison of results on Dorset's and Blacklock's media on this occasion do not reveal any real significant difference ($P = 0.0134$) and tends to support the contention that little or no real difference exists between the various media employed, the simple ones generally being as efficient as the more complicated media, although in this instance material (basal exudate), probably containing many more tubercle bacilli per unit volume than material (cerebrospinal fluid) dealt with in the previous experiment, was employed.

TYPE DETERMINATION OF ISOLATED ORGANISMS.

Human and bovine tubercle bacilli possess certain characteristics which make it possible to differentiate them quite definitely in most instances.

These characteristics are the following:-

Morphology.

As a rule the human type of bacillus tends to be long, thin and curved and to show granular staining. The bovine type on the other hand tends to be shorter, straight and thick with more uniform staining. Their morphology, however, is so variable and is so dependant on environmental factors, that no reliance whatsoever can be placed on these characteristics as a means of distinguishing the types. This has been generally accepted by all investigators, and has been completely ignored in this investigation.

Cultural Characteristics.

Smith (1896) was the first to note that tubercle bacilli isolated from human sputum grew freely on serum while organisms isolated from cattle grew very sparsely on that medium.

Dorset (1902) found that on the egg medium devised by himself similar differences existed between the bacilli isolated from these two sources. Group 1 (bovine type) showed scanty growth, and in cultures from the guinea pig the colonies were small, flat and tended to coalesce. The growth in fresh cultures was moist, glistening, and could easily be brought into a state of fine subdivision in bouillon. Group 2 (human type) in original cultures showed round, elevated colonies, and in subculture the growth was more abundant than in group 1, and could not be broken up so easily, being also more adherent to the medium.

The sharpest separation into different types,
however/.....

however, was made by Kossel, Weber and Heuss (1904; 1905) who, by using glycerine bouillon as the differentiating medium, noted that the one type (human) grew luxuriantly and rapidly while the other type (bovine) grew sparsely, more slowly and unreliably.

This was the first time that glycerine had been used in a differentiating medium, but the results were soon confirmed by many workers including the British Royal Commission on Tuberculosis (R.C.T., 1907a).

The conclusions as to the cultural characteristics as given by the Royal Commission are somewhat complicated by the varying methods employed by its different investigators, but in general, basing conclusions only on the amount and rapidity of growth, they conclude that all cultures fall into two broad classes - a dysgonic or poorly growing class, and a eugonic or good growing class (R.C.T., 1907b). They also state that differences in growth become more marked on media containing glycerol, as growth of early cultures of the first group is little if at all aided by glycerol while members of the second class usually flourish more luxuriantly than ever (glycerophilic).

They go on to state, however, (R.C.T., 1907c) that variations do occur and that the better growing cultures of the first class may differ very slightly, or approximate completely, to rather poorly growing cultures of the second class. Thus while this method of differentiation can possibly be relied on in the vast majority of cultures, because of the above mentioned variants, erroneous results can be expected if sole reliance is placed on this method.

Another cultural characteristic to be considered as a possible means of differentiation is the property of pigmentation. Arpad (1904) stated quite definitely

that/.....

that the formation of an orange to brick red pigment on potato was a distinctive characteristic of the human type of bacillus.

Later investigations have, however, found this very irregular in appearance (Park and Krumweide, 1910a; Blacklock, 1932b) and not invariably correct (Park and Krumweide, 1910a) as these investigators found some of their bovine strains to be similarly pigmented. Novick (1919) also describes a pigmented bovine strain in his series of meningitis cases.

On other media (serum or egg) pigmentation is even more variable. Griffith (1916), using inspissated bovine serum found pigmentation to be very characteristic of the human variety, but subsequent workers like Blacklock (1932b) found it to be very variable and to depend largely on the particular serum used.

Egg media are even less reliable as many human strains fail to produce pigment on such media, although Park and Krumweide (1910b) do report some success with it. Blacklock (1932b) however reports some of his bovine strains as showing pink pigmentation on egg media.

The above can be summarised by stating that although definite pigmentation (especially on potato) is suggestive of a particular strain being of the human type, this result should be correlated with results of other typing experiments before the organism is finally classed as belonging to one or other of the types.

Another possible differentiating feature between the two types was discovered in 1904-5 by Theobald Smith who published his observations on a characteristic difference in reaction curves obtained when the human and bovine types of tubercle bacilli were grown in glycerine broth with an initial acid reaction to phenolphthalein. The bovine tubercle bacillus during growth

gradually/....

gradually decreased the acidity of the medium until finally the reaction was about neutral. The human bacillus on the other hand, after initial alkali production, soon caused the medium to revert to its initial low pH. That is to say, the final reaction of the bovine culture was about neutral whereas that of the human cultures was decidedly acid.

Smith's view briefly was that the human type was able to utilize glycerol with consequent production of acid, whereas the bovine bacillus was unable to use it, and so caused an increased hydrolysis of protein and an alkaline reaction. However the reaction curve of broth cultures is determined by so many factors, such as the production of ammonia, carbon dioxide, amino acids and other substances some of which are volatile, that it is almost impossible to determine whether the production of acid is due to disintegration of the glycerol by the bacilli or to hydrolysis of protein material.

The general opinion regarding this test is that as a rule the reaction curves of the two types differ exactly as Smith claimed, but that this difference is not to be explained on any fundamental physiological difference (Harden, 1913) in the metabolism of the two types, such as a capacity, or want of a capacity to utilise glycerol and so form acid from it as Smith maintained, but rather that these differences are simply related to the different rates and quantity of growth of the two types of organism (R.C.T., 1911a).

Thus such differences of reaction as are observed only serve in effect to give information of the same kind as can be obtained more easily by observing the rate and quantity of growth on various solid media.

When the technical difficulties and the time (up to
3 months/....

3 months) required for this test are considered, it is small wonder that very few investigators have employed it at all, and certainly none have used it during the last two decades. It has been completely disregarded in the present investigation.

Antigenic Structure.

Although Schaeffer (1940) claimed to have isolated a protein antigen specific for the bovine type of bacillus, there is as yet no recognised serological method of distinguishing between the two types of mammalian tubercle bacilli (Bergey, 1948; Topley and Wilson, 1948c), and there have been no reports of type determination by such methods.

Of the various differentiating tests mentioned above, only those of growth and pigmentation on solid media are generally employed, and various technical details concerning these tests will now be discussed.

Accepting the fact that the most constant difference noted so far between the two types is in their relative amounts of growth, the problem is how best to demonstrate this disparity.

It is freely conceded that there is a slowly increasing luxuriance of growth evident in most strains upon repeated subculture in the laboratory resulting from a progressive adaptation to an artificial existence. (Park and Krumweide, 1910c; Cobbett, 1917a; Blacklock, 1932c; and Topley and Wilson, 1948a).

The question therefore arises at what period of saprophytic life are cultural type characteristics likely to be most distinct.

It might be thought that the most favourable period

for/.....

for bringing out the differences in the cultural characteristics of the different types would be when they are still recently isolated from the animal body and before adaptation to the new conditions of saprophytic existence has had time to blunt the points of distinction. But, as Topley and Wilson (1948a) point out, both human and bovine bacilli are equally strict parasites and both require a certain time to develop their representative capacities for growing on artificial media: moreover, in each type different strains adapt themselves to artificial media with different degrees of rapidity and because it is not so much the readiness with which a given strain will grow on artificial media at the start which gives us the desired intimation of the type of the strain, as the character and the degree of luxuriance of growth when once the preliminary difficulties of adaptation to a new environment have been overcome. It is for this reason that it is probably less easy to judge the type of the strain from first generation cultures. However, although Cobbett (1917b) maintains that there is no need to hurry the examination, most investigators prefer to proceed with the investigation on the first, or more frequently, the second or third generations, noting that the distinction tends to become less marked when later generations are used.

There is, however, complete unanimity about the fact that isolation of the organism must be made on glycerol free media, and all subcultures for typing purposes done from these media. This is so because the bovine bacillus can adapt itself very well to glycerol and eventually come to be quite eugonic in character.

The next question which arises, is at what age are the characters of a particular culture most clearly marked; in other words, is it the rapidity of growth or the quantity of growth which is most important to determine.

Continued/.....

Continued growth beyond 3-4 weeks is not marked with eugonic cultures, while most dysgonic strains continue growing slowly beyond that time (Cobbett, 1917f). According to Cobbett though, the rate of growth is a more variable factor than ultimate quantity produced and he regards the latter as the most important feature. Consequently he prefers to set no particular time limit at which cultures should be read for amount of growth, and judges each case individually.

Park and Krumweide (1910d) however, set a time limit of 3 weeks for growth to occur on their cultures, i.e. they do take rapidity of growth into account. They admit, however, that comparing the growths a week later (4 weeks instead of 3 weeks) did not make any appreciable difference to results obtained.

Finally, the question remains as to what medium is the best to use for eliciting these differences in growth. The ideal medium would have the following properties:

- (1) It should be uniform in its results, i.e. growth should not fluctuate with different batches of media used.
- (2) It should be especially adapted for the growth of eugonic strains so that the best possible growth these strains are capable of is brought out.
- (3) It would be preferable if growth of dysgonic strains were somewhat retarded on this medium as this would widen the gap between dysgonic and eugonic types and facilitate the interpretation of results.

With regard to the first point, in the absence of a suitable synthetic medium, the simplest possible medium would be expected to yield the most reproducible results, and Dorset's medium and a potato medium immediately suggested themselves. Two media were used, for as Cobbett (1917g) suggests, the determination of cultural characteristics is not so easy that one can afford to lose any information that might be gained by widening

the/.....

the range of media employed.

The potatoes and eggs used in these media are of course variable factors as their compositions are subject to changes, but a certain amount of control can be exercised on the latter by obtaining fresh eggs from the same source of supply.

As regards the second and third points mentioned, glycerol is the obvious substance to incorporate in the media, for while it has a definite stimulating effect on the growth of most human types, it usually has a restraining effect (or at the most a very slight stimulating effect) on bovine types. Thus with typical strains at least, differences in growth should be maximal.

The media used for type determination are thus the following:

- (1) Dorset's egg medium prepared in exactly the same way as for isolation purposes.
- (2) Dorset's medium with glycerol added to make a final concentration of 5%, prepared in exactly the same way as the previous medium.
- (3) Glycerine potato medium prepared according to the method employed by Park and Krumweide (1910k). Fresh potatoes were cut into cylinders by means of a cork borer with a diameter slightly smaller than the diameter of the potato tubes used. The cylinders were then cut obliquely in half, and the halves soaked in 1:1000 sodium carbonate solution for about 24 hours. After this the halves were placed in the potato tubes which were then filled with a 5% aqueous solution of glycerol and placed in an Arnold steam steriliser for 30 minutes, after which all but the glycerol solution remaining in the bulb of the tube was poured off, cotton-wool stoppers replaced, and the tubes autoclaved at 10 pounds pressure for twenty minutes.

After inoculation, the cotton-wool stoppers of the tubes were sealed with paraffin wax.

This medium has the advantage that, apart from giving the usual growth differences, pigmentary tendencies of strains are usually brought out maximally.

Growth of bovine strains on these media (although subject to definite variations) are characteristically

poor/....



Fig. 2. Grade 3 growths of tubercle bacilli



Fig. 3. Grade 8 growths of tubercle bacilli

poor and sparse and usually lacking in any pigment. Dysgonism is possibly best brought out on the glycerine potato medium and has been used with great success by Park and Krumweide (1910b); Griffith (1919-20); Blacklock (1932b); Griffith (1934); and Blacklock and Griffin (1935). Growth (especially on the egg media) tends to be rather moist and emulsifies much more readily than the human type.

The human variety in typical form is almost the exact antithesis of the above. It grows well on all media, especially media containing glycerol, and the dry heaped-up warty appearance, particularly on the potato medium, is very characteristic especially when pigmentation is present as well.

As far as cultural characteristics were concerned, the typing procedure was then as follows: as soon after isolation as possible first generation cultures which had never come into contact with glycerol were cultured (in the inoculation box) onto two bottles of plain egg medium, two bottles of 5% glycerol egg and one potato glycerine medium.

The egg media were incubated at 37 degrees Centigrade for 3 weeks in an upright position after the addition of about 1 cc. of sterile saline to prevent drying out of the medium.

For ease of recording cultural luxuriances, numerical grades of growth as used by Park and Krumweide (1910d) were adopted. Nine grades were arbitrarily selected; thus grades 1-3 sparse; grades 4-6 moderate; and grades 7-9 luxurious, as illustrated in figures (2) and (3) on the opposite page. The total amount of growth in the tubes was simply judged by inspection of the cultures,

and/....

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and/....

and as minor variations occur at times in the individual tubes of a set of transfers, it was the rule to select the best growing tube of each pair of cultures for recording the grade. It is only reasonable to consider (as Park and Krumweide, 1910d, and Blacklock, 1932c, suggest) that the best tube is an index of the capacity of the organism to grow under the most favourable conditions.

Glycerine potato tubes were incubated for at least a month and then left on the bench at room temperature for a week or two before being read for the amount of growth and the presence or absence of pigmentation. Growth was judged by inspection of the cultures and recorded numerically as with the egg media.

In the event of any of the cultures being contaminated, the experiment was immediately repeated. This was found to be necessary on six occasions.

As isolation was attempted on glycerol and glycerol free media, in the event of growth being present on both types of media, an indication could often be obtained as to the type of the particular organism by comparing the growths on these media. Judgment of this kind (on first generation growths) was, however, never final and the procedure outlined above invariably carried out.

Virulence Characteristics.

Although early investigators (Villemin, 1868) had noticed differences in virulence for animals of tuberculous material derived from humans and cattle, it was Smith (1896; 1898) who first attempted to correlate these differences with results of cultural tests noted in tubercle bacilli derived from human and bovine sources. Since then numerous other workers (R.C.T. 1907d) have confirmed these findings that bovines, goats, sheep, pigs
and/.....

and rabbits, while very susceptible to bovine infection, are relatively resistant to infection with the human type of bacillus.

Here, then, is another method of possible distinction between types of tubercle bacilli, and all that remains to be discussed are various technical details.

What is the animal in which these differences of pathogenicity will be most manifest? The most extensive investigation performed with this point in mind was carried out by the British Royal Commission (1907d) who came to the conclusion that "the results produced by injecting the bovine tubercle bacillus into calves and rabbits in the above doses are thus very striking and definite, and taken with the cultural characters of the bacillus, afford a trustworthy means of recognising the bovine bacillus. Indeed, as our investigation progressed, we found that it was sufficient to inoculate the rabbit for differential diagnosis" (R.C.T. 1911g).

Park and Krumweide (1910e), amongst others, working along the same lines also found the rabbit to be perfectly satisfactory for diagnosis of type. The calf and the rabbit have the important advantage over other animals in that they very rarely contract natural tuberculous infection, and while the calf is considered just as reliable as the rabbit, its greater cost taken in conjunction with grazing and stabling difficulties, have made all subsequent workers rely on the rabbit to the exclusion of all other animals.

Accepting the rabbit then as the most satisfactory animal, what is the most suitable route of infection and dose of organisms to employ? It must be remembered that the rabbit is definitely more susceptible to infection with the human type of organism than the calf or goat and consequently the infecting dose must be more strictly controlled than in the case of the latter two animals

mentioned/....

mentioned. This immediately excludes two routes of infection which have been employed for calves and goats, namely feeding and inhalation, as apart from the danger to the operator, the dose of bacilli cannot be accurately controlled.

Fraser (1912) and Harvey-Pirie (1932) in this country have employed an intra-articular route; a method by which 1 cc. of a suspension of organisms is injected into the synovial cavity of the knee joint of the rabbit, and the test is judged on the degree of disability resulting. According to them the human type produces at most a transient disturbance of function while the bovine bacillus eventually leads to total disorganisation of the joint and dissemination of the infection.

This method has, however, not been generally accepted and most workers have employed either the intravenous, subcutaneous or intraperitoneal routes of introducing infection for type determination.

Using the intravenous method, it must be borne in mind that progressive disease, eventually resulting in death, may be caused in rabbits by human bacilli in adequate numbers especially by this route, and although a dosage of 0.01 mg. gives good results (R.C.T., 1911c), slight increase in the amount often leads to equivocal results. In favour of this method, though, is the fact that by employing the correct dosage (0.01 mg.) results can often be obtained in a month to six weeks.

As regards the other two routes, the question of dosage is not nearly as important, and with regard to the subcutaneous route, Cobbett (1917c) actually goes so far as to state that human tubercle bacilli introduced subcutaneously in any dose, never produce a progressively fatal form of disease in the rabbit.

The usual subcutaneous dose employed is 10 mg. (R.C.T., 1911c).

Cobbett/....

comes to the conclusion "that attenuation under artificial cultivation is as a rule very slight, and often imperceptible after one or more years, and that when it occurs it does so capriciously, and would seem to be determined by causes about which at present little is known".

Thus, while this view is generally accepted, definite attenuation of cultures has been noted by many workers including Park and Krumweide (1910f) who noted its occurrence in two of their bovine strains, and the general tendency has been for workers not to delay unduly the doing of virulence tests.

The question whether growth for virulence tests should be obtained from glycerol free media or not, is apparently unimportant (R.C.T., 1907e) and many workers including Park and Krumweide (1910g) and Blacklock (1932d) prefer the use of glycerol containing media, as growth of the human type is usually more readily emulsifiable when derived from such a source. They also used growth obtained from glycerol free media and noted no difference in results obtained. All workers are however agreed upon the fact that it is essential when doing virulence tests to employ young and vigorously growing cultures obtained by using subcultures not more than 3 weeks old.

The methods used in this investigation are the following:

An attempt was made to do the virulence tests as soon after isolation as possible, but due to lack of accommodation, this was rarely possible before a month at least had elapsed.

At least 10 mg. of vigorously growing subculture (mostly on glycerine containing media) of between 10 and 14 days old, were injected subcutaneously into the outer surface of the right thigh of one rabbit weighing at

least/.....

least 1200 gm. Only one rabbit was used for each case.

After 3 to 4 months the rabbit was killed, a post-mortem examination performed and results recorded. Rabbits dying before that time were similarly dealt with. Due attention being paid to the possibility of various non-tuberculous conditions simulating tuberculosis, only macroscopic lesions were recorded, and like Park and Krumweide (1910h) only the power of the organism to cause progressive disease was considered - the presence, number, distribution i.e. the degree of generalization of the macroscopic lesions were assumed a fair index of this power.

Whenever a rabbit died before the set period (3-4 months) with no tuberculous lesions to account for death, the test was repeated.

In addition to this route of infection, growths considered to be of bovine type on cultural characteristics, were inoculated into two more rabbits, 0.01 mg. intravenously and 1 mg. intraperitoneally, respectively.

For each case, too, a guinea pig was inoculated simultaneously with a suspension of the organism amounting to about 0.2 mg. intramuscularly into the right thigh to detect avian type bacilli and ensure that pathogenic and not saprophytic acid fast bacilli were being dealt with.

If not dying earlier, these guinea pigs were killed after 6-10 weeks had elapsed, and examined for tuberculous lesions as described in the section dealing with isolation of the organisms.

Technically, the preparation of the correct dose of tubercle bacilli for injection is a most difficult task. Due to the nature of the material, direct weighing of the organisms to the correct weight is hardly ever attempted, and/.....

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and the usual procedure is to weigh out more than the required weight, suspend it in a known volume of fluid and use proportionately less to get the exact weight required. This weight-volume relationship of course only holds good if the organisms are perfectly dispersed. Due apparently to their high lipoid content, these organisms are exceedingly difficult to disperse in water and various surface active agents have been employed to give better dispersion. But while excellent dispersion is usually obtained by these methods, there is no knowing what effect these agents have on the virulence of the organisms and most workers have therefore simply used water or saline as emulsifying agents. Thus solutions of sodium taurocholate, Tween and albumin and even serum produce very even suspensions, but they were not used in this investigation in order to make results obtained strictly comparable with those of previous investigators.

As in most other investigations, moist weights were used - i.e. the tubercle bacilli were scraped off the medium and weighed at once without the intermediate step of drying them between sterile sheets of blotting paper.

Bovine bacilli usually emulsified remarkably well but this could not be said for the human variety, although use of cultures from glycerine containing media facilitated the process slightly.

The procedure was then as follows: a sterile stoppered graduated 15 cc. centrifuge tube was accurately weighed on a Baird and Tatlock Aperiodic Balance to four decimal places. Working in the inoculating box, approximately 10 mgs. of growth were scraped off the medium by means of a wire loop and deposited at the bottom of the weighed centrifuge tube which was restoppered and weighed as before.

The weight of the tubercle bacilli could then be
calculated/.....

calculated. With a little experience it soon was possible to estimate the amount of growth to within 2 or 3 mgs. of the correct weight, and if on weighing the result was found to be of this order, i.e. 12-13 mgs., all of it was inoculated subcutaneously into the rabbit after emulsification. Thus by far the majority of rabbits received more than the required dosage of 10 mgs. subcutaneously.

When, however, the weight exceeded 15 mgs. the organisms were ground up in the graduated centrifuge tube in the inoculating box for ten minutes with a close fitting pestle. After this period of time a drop of sterile water was added and further grinding proceeded with. More drops were then gradually added until after 30 minutes' grinding the organisms were in a fairly reasonable state of dispersion in about 2-3 cc. of water. After reading this volume accurately, the volume of water containing 10 mgs. was calculated and injected subcutaneously into rabbits. When 1 mg. and 0.01 mg. dosages were required, the initial quantity weighed was much less, and if necessary, the volume of fluid added was much larger so as to enable the desired weight to be contained in 1-3 cc. of the water - a convenient volume for injection purposes.

As Corper and Cohn (1933) stress, the essential step in the above procedure is grinding of the bacilli initially for some period of time without the addition of any water, and it was found that if this method was strictly adhered to, reasonably well dispersed suspensions were always obtained.

The question now arises as to which characteristic is the most important, i.e. if divergent results are obtained on cultural and virulence tests, on which one is the final decision as to type going to depend?

Opinions/.....

Opinions have differed on this point, and even Griffith (1914) working with lupus strains firstly stressed cultural characteristics as being the most important, but later (Griffith, 1929) modified his opinion and believed that more reliance should be placed on virulence tests. At present this latter view, i.e. whether or not a particular organism is capable of causing progressive disease in calves or rabbits, is generally accepted as most important, although the only sound procedure is to assess the type only after reviewing all its characteristics carefully, and this is usually done.

Fortunately this problem does not often arise, and in the vast majority of cases a definite inverse relationship exists between the amount of growth on solid media and rabbit virulence. Great prominence has, however, been given to discrepancies between the two tests by workers who believe in the essential unity of the tubercle bacillus and regard these disparities as proof of their claims.

They believe that all types are essentially the same and that the differentiating features mentioned above are simply acquired through prolonged residence in a particular host. In other words, that a human tubercle bacillus has certain features simply because of prolonged residence in human tissues and that it can be converted to what we now call the bovine bacillus by passage experiments in that animal.

The facts, however, are that this has never been observed experimentally, and while freely conceding that the virulence of an attenuated bovine strain can sometimes be restored by suitable passage experiments, Park and Krumweide (1910i), R.C.T. (1911b), Cobbett (1917e), Griffith (1932a) and Topley and Wilson (1948b) maintain that the particular type of bacillus is never changed.

This/.....

This has been confirmed by Fridmont-Möller (1939) who published an extensive monograph on the subject. Recently Jensen (1949) has revised his earlier opinion (Jensen and Fridmont-Möller, 1936), and now regards the occurrence of mixed infections as not necessarily the result of cross infection, but sometimes the result of transition of types.

The variants usually encountered are (1) strains which grow poorly, possibly producing pigment and giving typical human results on rabbit inoculation. These Griffith (1924) classifies as dyegonic human in type. An additional variation rarely encountered with strains like the above is for virulence to be attenuated for the guinea pig. (2) Strains which grow rather well (rarely quite as well as the typical human strains though), usually produce no pigment, but are highly virulent for the rabbit. These are classified by Griffith as being eugonic bovine strains. The perplexing cases, however, are those (3) which grow fairly well, like the one just mentioned, but instead of having the standard bovine virulence, have an intermediate effect on rabbits. The virulence of such strains can often be restored by passage experiments, and most workers classify them as being eugonic bovine in type, especially if they produce no pigment.

Definite eugonic cultures (i.e. of a luxuriance of growth exceeding the eugonic bovine strains mentioned above) proving virulent for the rabbit are invariably mixed strains, i.e. bovine bacilli are present as well (Griffith, 1914).

It must, however, be repeated that such strains as those mentioned above are rare, and although they have been recovered from sputum (Griffith, 1916), bones and joints/...

joints (Griffith, 1916-17) and cerebro-spinal fluid (Griffith, 1932b), most of them are confined to cases of cutaneous tuberculosis.

Seeing then, that the vast majority of cultures conform strictly to type, the question arises as to the necessity of doing all the tests mentioned routinely for type determination.

Morphological, antigenic and reaction curve tests have already been described as unreliable or redundant, and have been disregarded by all workers conducting statistical surveys regarding the incidence of bovine infection.

Cultural tests are relatively easy and cheap, but the same cannot be said for virulence tests which, in addition, are time consuming. Thus while cultural tests are invariably done, the tendency has been, especially in large statistical surveys, to limit the virulence tests to selected cases only.

Thus Blacklock (1932e) did not test the virulence of 58 eugonic strains classified as human in type; Griffith (1928; 1934) and Griffith and Smith (1935) during investigations on bone and joint, meningeal and pulmonary tuberculosis respectively only tested dysgonic cultures for virulence; and Park and Krumweide (1910j) came to the conclusion that "all cultures showing maximum luxuriance on glycerine egg in primary cultures are surely of the human type" and do not recommend virulence tests for such cultures. Nor do they advise virulence tests on typically dysgonic cultures and are prepared to accept these as being bovine in type. In fact the only use they have for the rabbit in the scheme they recommend is for virulence tests on growths of intermediate character, and maintain that the error introduced in large statistical surveys/....

surveys by following their scheme will amount to less than 1%.

This has been accepted by most authorities and the National Surveys of England and Wales (1943-45), and Scotland (1943-44) were conducted along these lines.

Reilly (1950), working in North Ireland, followed a similar course and Kearney et al. (1949) who examined 500 cases of pulmonary tuberculosis only did rabbit virulence tests on dysgonic cultures.

Webster (1941), and Finlayson and Edson (1947), investigating extrapulmonary tuberculosis in Australia (Victoria) and New Zealand respectively, followed similar procedures.

While agreeing with Park and Krumweide, and Webster that a culture growing with maximum luxuriance and showing pigmentation in the first or second generations "is surely of the human type", I must mention that luxuriance of growth is not measured absolutely, and is therefore liable to different assessments, and eugonic bovine cultures may be misinterpreted especially by inexperienced workers. Mixed infections may be missed altogether, although this consideration is not relevant as far as this investigation is concerned, as mixed infections have never been described in a peripheral focus such as the meninges.

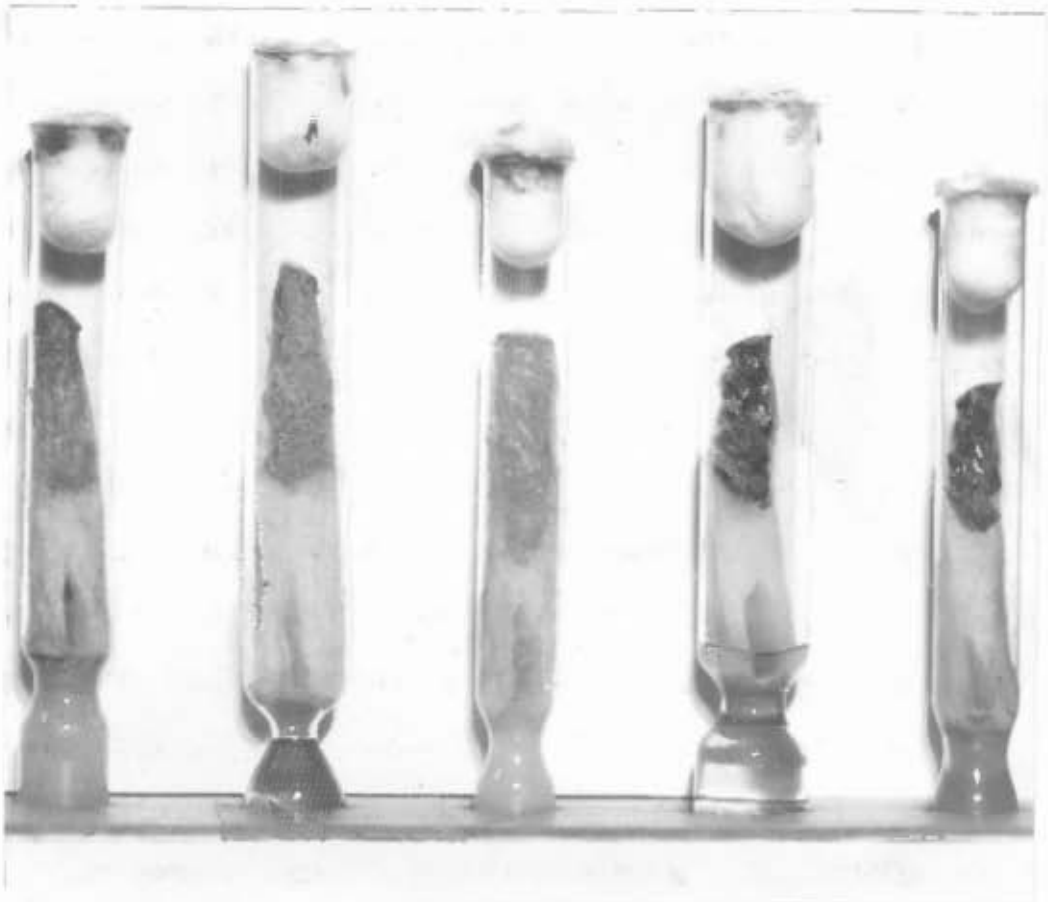
Most workers have, however, tested the virulence of their dysgonic cultures for fear of misinterpreting dysgonic human types.

In this investigation I have preferred not to deviate from the standards laid down by the Royal Commission and have therefore submitted all bacilli to both cultural and virulence tests.

Results.

Results of cultural and virulence tests agreed so closely/.....

Fig. 4. Pigment Production of Human Tubercle Bacilli on Potato.



closely that they can conveniently be discussed together.

Firstly, all strains typed caused manifest and disseminated disease in guinea pigs and must therefore be considered pathogenic (as distinct from saprophytic) and possessed of a normal degree of virulence for that animal. No possible avian types were thus encountered.

Results of typing 198 of the 200 different strains from the cerebro-spinal fluid, and 17 of the 18 strains isolated from caseous mesenteric lymph nodes, can be stated quite briefly as they were all typically human in type.

They all grew to a grade 5 or more in the second generation on glycerine free media, and were all glycerophilic in that second generation growths on the glycerol egg media were always better, grades 7 to 9 invariably being registered. Second generation growths of these strains on the glycerol potato medium were even more distinctive in that every strain grew luxuriantly, (grades 6-8), eventually producing a thick wart-like dry growth, and practically all produced a reddish shade of pigment on this medium, as is shown in Figure 4 on the opposite page.

Rabbit virulence tests were equally conclusive, not one rabbit dying of tuberculous disease during the period of observation (3-4 months).

At autopsy these rabbits either showed no lesions or scanty, well circumscribed and obviously retrogressive lesions in the lungs and in a few cases also in the kidneys. In ten cases there was ulceration at the site of inoculation.

Rabbits inoculated with material from seven of these cases died during the period of observation, and at autopsy showed no tuberculous lesions. Fresh rabbits inoculated subsequently with these strains were perfectly fit at the end of four months and at autopsy showed the usual minimal evidence of tuberculosis.

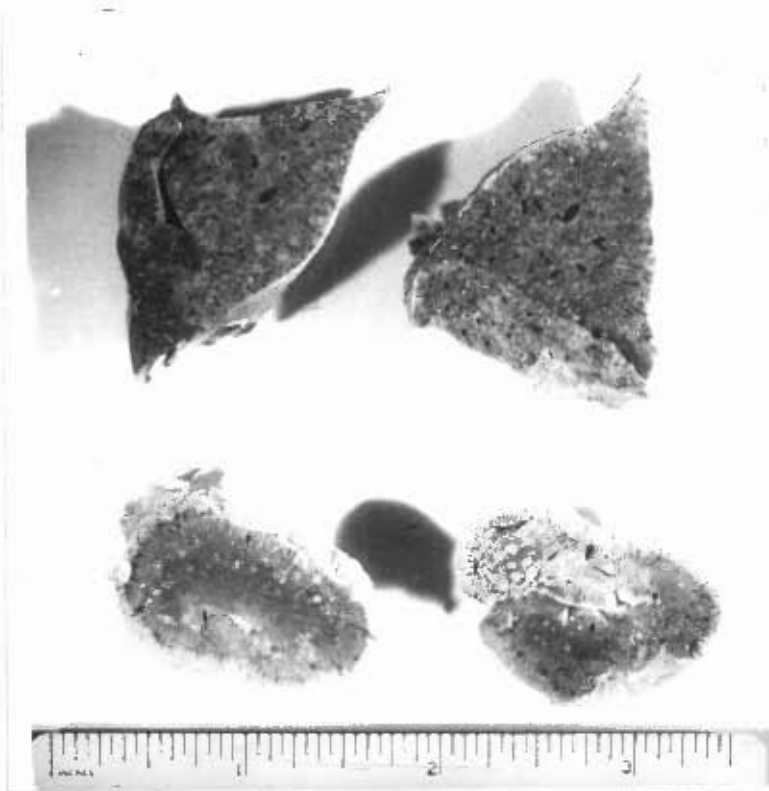
As regards the remaining two cases of tuberculous meningitis, strains of organisms were isolated from the cerebro-spinal fluid and caseous mesenteric lymph nodes in the first case, but only from the cerebro-spinal fluid in the second case as no post mortem examination was performed.

Both strains from the first case were typically bovine in that they produced only a grade 3 growth in the second generation on plain egg and grew even more sparsely on glycerol egg.

These growths were greyish in colour and tended to be slightly moist. Characteristically second generation plantings on glycerol potato hardly succeeded in growing at all, eventually producing no more than a greyish, slightly moist grade 2 growth. Rabbits inoculated intravenously, intraperitoneally and subcutaneously with strains derived from both sources, died in 25, 35 and 54 days and 30, 37 and 60 days respectively with severe generalised miliary spread of the infection as illustrated in Figure 5.

FIGURE 5.

Lungs and Kidneys of rabbit inoculated with organisms from one Bovine Case showing Miliary Spread of Infection.



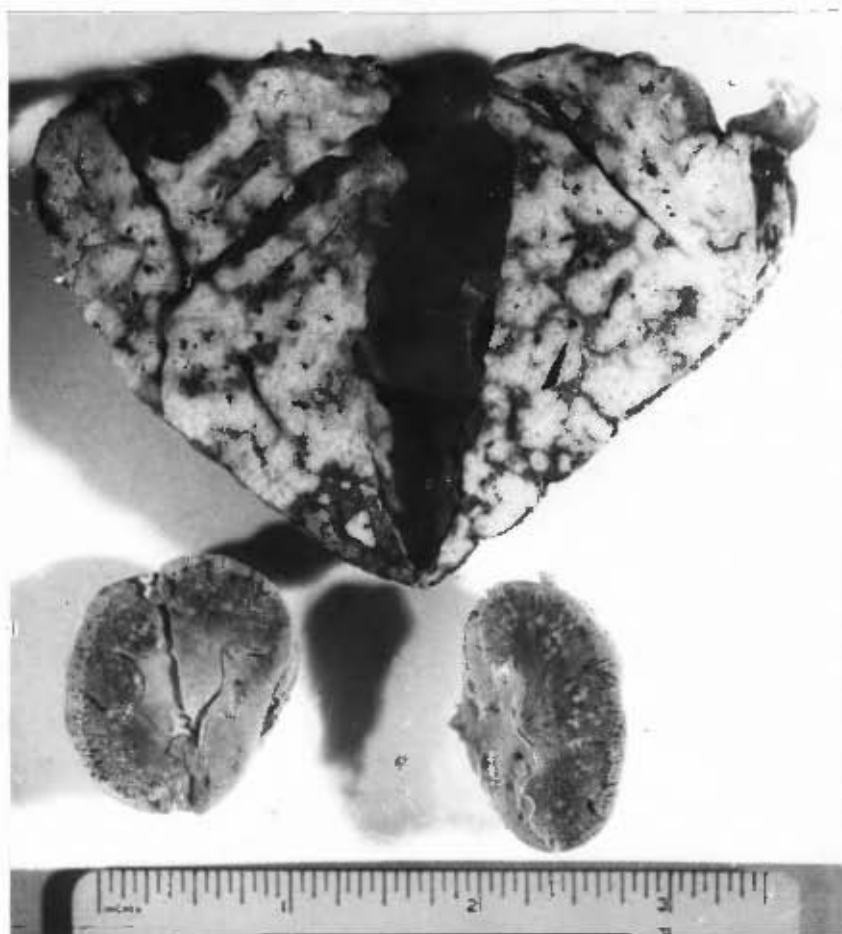


Fig. 6. Lungs and Kidneys of rabbit inoculated with organisms from second bovine case showing extensive spread of disease.

The second case was less typical in so far that it repeatedly produced a grade 5 growth on glycerol free egg, but grew less freely on glycerol egg, second generation plantings on this medium producing no more than a dysgonic grade 3 growth.

Growth on glycerol potato was equally dysgonic, a grade 2 growth being the best growth registered. It differed from the bovine case just mentioned, in producing a suggestion of a reddish pigment on this medium.

The organism was, however, fully virulent for the rabbit. Animals injected intravenously, intraperitoneally and subcutaneously with this organism died on the 30th, 42nd and 51st day after inoculation, with a severe generalisation of the disease as illustrated in Figure 6 on opposite page. This organism was then also judged to be bovine in type.

It remains to be stated that two strains of tubercle bacilli isolated from cow's milk proved typically bovine in type as judged on the same typing experiments described above.

Thus out of a total of 200 cases of tuberculous meningitis examined, the bovine type of bacillus was present in only two instances - i.e. 1% of cases.

EPIDEMIOLOGY OF TUBERCULOUS MENINGITIS

In this section the general epidemiology of tuberculosis will be discussed, and the results of the investigation into the environment of the 200 cases examined bacteriologically will be correlated with other information in an attempt to substantiate the results of the typing experiments.

The two varieties of tubercle bacilli to be discussed are the human and the bovine types as they are almost solely responsible for disease in man. The avian type may be dismissed owing to the great rarity of this type of infection in the human subject (Gloyne, 1933; Feldman, 1938; Bradbury and Young, 1946) and because it was not encountered in this investigation.

Without citing any of the extensive literature brought to bear on this particular problem, it can be stated quite categorically that, apart from the rare congenital form of transmission, the human type of bacillus passes from man to man only by droplet and dust infection, and it is for this reason that infection with this type of bacillus is usually, though by no means invariably, respiratory in nature and tends to occur most frequently where susceptible and tuberculous individuals come into close and repeated contact with one another.

Infection with the bovine type of bacillus is found chiefly in cattle, though many other types of animal, including man, are liable to infection.

The chief mode of spread amongst cattle is also by means of droplet infection, and thus, as in man, the primary lesion in bovines is also most frequently found in the respiratory system (Francis, 1947; Blacklock, 1947).

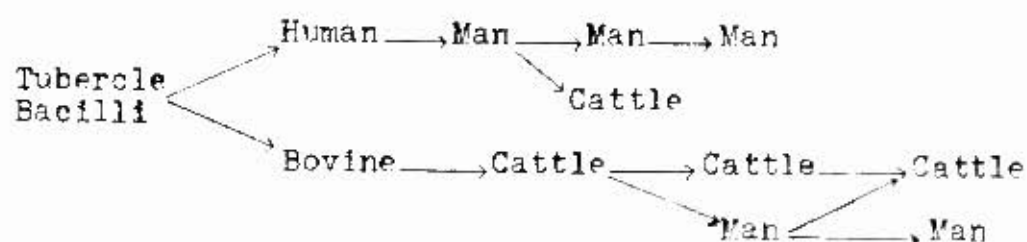
Man is infected with bovine bacilli chiefly through ingestion of contaminated milk (Blacklock, 1932j; Cumming and/....

and Foster, 1933; Griffith and Munro, 1935; Griffith and Smith, 1935; Griffith, 1937; Lange, 1937), though airborne infection is also possible, as is well exemplified in the cases of bovine conjunctival tuberculosis in Danish milkers reported by Jensen et al. (1940) and pulmonary tuberculosis reported by Cutbill and Lynn (1944) and Sigurdsson (1945). The cycle of infection, however, does not stop there, as it has been observed that bovine infection may pass back from man to cattle (Nielsen and Plum, 1939; Plum, 1940; Magnusson, 1941, 1942; Griffith and Munro, 1944; Tice, 1944), and also from man to man, as in the five cases reported by Griffith and Munro (1935), Munro (1939), Jensen et al. (1940) and Hedvall (1942).

The human type of bacillus may also infect cattle, as it has been observed that cows in contact with human cases of pulmonary tuberculosis, though not developing signs of active disease, may give a positive tuberculin reaction, and the human type of bacillus has actually been isolated from such cases (Reid, 1939; Feldman and Moses, 1941). The human type of bacillus has, however, never been isolated from the milk of any cow. This information is summarised in Figure 7. (after Blacklock, 1947).

Fig. 7.

CYCLE OF INFECTION IN MAN AND IN CATTLE



The chief pathway of infection for the human subject is via the respiratory system. This has been well brought out/.....

out by a series of 434 autopsies performed on Scottish children by Blacklock (1947) who found the primary site of infection to be in the respiratory tract in 65.2% of cases, in a country where human bovine infection is possibly the highest in the world.

Also that this respiratory infection is predominantly due to the human type of bacillus is shown by the fact that Blacklock (1947) isolated the human type of bacillus from 96.4% of 166 cases of respiratory tuberculosis in Scottish children under 13 years of age.

Respiratory infection is most often caused by the inhalation of infected droplets; occasionally it may be due to tuberculosis elsewhere, as, for example mesenteric or cervical lymph nodes, the organisms usually reaching the lungs by blood spread from those sites.

Tubercle bacilli must, however, frequently be inhaled without producing any recognisable lesion, for tuberculosis is a universal disease making the chances of infection enormous, and what prevents the disease from assuming epidemic proportions must simply be the native or acquired resistance, or both, of individuals, although the true nature of these factors is still obscure.

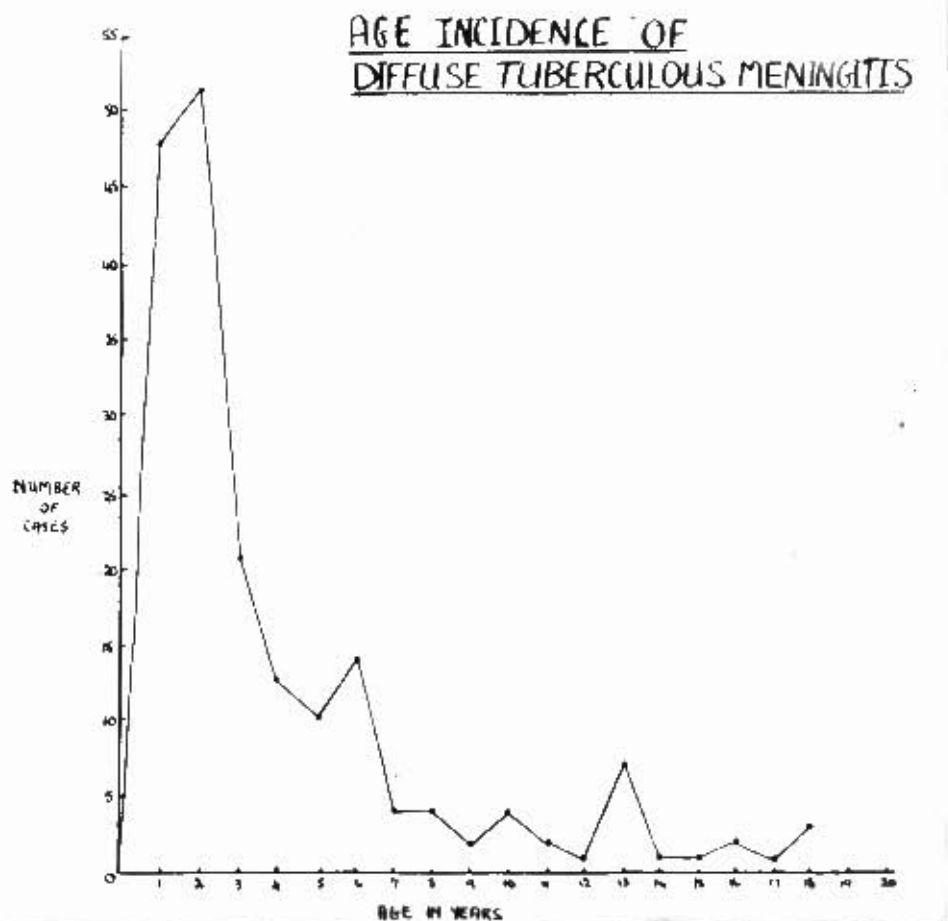
What, however, is known, and has been abundantly proved, is the fact that it is possible to break down these powers of resistance, be they natural or acquired, by exposure to massive doses of tubercle bacilli, by the presence of other diseases, by malnutrition and by overwork. Add to these the factors of overcrowding and ignorance of the disease, and we have the exact position reigning in the homes of the vast majority of the patients investigated in this work.

If the primary lesion does not remain localised at or near the site of infection then dissemination occurs resulting in a generalisation of the disease with or without a meningitis.

Tuberculous meningitis being a metastatic (i.e. secondary) phenomenon to the infection in the lungs or
alimentary/.....

alimentary canal, its occurrence implies some failure of localisation at those sites and the frequent association of meningitis with an active primary complex has been attributed (Rich and McCordock, 1933; Rich, 1944) to lack of sufficient acquired resistance. Associated with this, of course, is the fact that young children being relatively immobile, if exposed at all, are usually exposed to massive and repeated household infections. This, in conjunction with the frequent debilitating diseases encountered in the young (which have a definite, though as yet unexplained effect on resistance) and the possibility of native resistance being less at that age, help to explain the disproportionately high incidence of meningitis in the very young. The following graph (figure 8) summarises the age incidence of the 200 cases studied and shows that exactly half the cases occurred during the first 2 years of life.

FIG. 8



Differences in the sex incidence have been commented upon (page 9) and although females predominate slightly in this series, no real difference exists in larger series of cases/.....

TABLE XV
SEASONAL INCIDENCE OF TUBERCULOUS MENINGITIS IN THE CAPE

Year.	NUMBER OF CASES PER MONTH.												Yearly total
	Jan.	Feb.	Mar.	Apr.	May	Jun.	July	Aug.	Sep.	Oct.	Nov.	Dec.	
1946	6	2	6	11	8	3	3	7	6	16	12	5	85
1947	10	7	7	4	6	8	7	17	9	11	15	10	111
1948	9	3	4	0	13	6	12	12	16	10	19	12	116
1949	8	12	16	14	9	9	16	20	17	14	11	12	158
1950	10	11	9	4	9	10	14	19	28	19	12	21	166
1951	8	18	25	13	9	13	5	14	28	20	11	15	179
Monthly total.	51	53	67	46	54	49	57	89	104	90	80	75	815

of cases (Schwarz 1948).

Table XV (on opposite page) indicates the monthly incidence of tuberculous meningitis cases diagnosed at the Union Health Laboratories, Cape Town, between the years 1946-1951.

A definite increase is noted in the late winter and spring months and is in conformity with the findings of Schwarz (1948) who, in an analysis of 11,332 cases reported in the literature, found the highest incidence to be in the later winter and spring months. This is possibly accounted for by the fact that confinement in poorly ventilated houses and rooms during the Winter accounts for more primary infections, which terminate as meningitis in the Spring. The lack of essential nutrients at the end of Winter and the frequent respiratory infections present at that time of the year tend to weaken the general resistance, and may also be factors accounting for the slight seasonal incidence of the disease.

Tubercle bacilli isolated from the cerebro-spinal fluid have been typed by innumerable workers in all parts of the world, and the human type of bacillus has invariably been encountered more frequently than the bovine bacillus, as will be seen from the following discussion on the rate of bovine meningitis in various countries.

In Scotland the overall incidence of bovine infection in meningitis calculated from the results of various workers published between 1907 and 1937 (Watt, 1907; Wang, 1916-17; Blacklock, 1932; Griffith, 1934; Blacklock and Griffin, 1935; MacGregor et al., 1935; Munro and Scott, 1936; MacGregor and Green, 1937) is 26%, the incidence rising to 36% in the series described by Munro and Scott.

During the National Investigation held in Scotland between the years 1943-44 a further 560 cases were typed, and in this series the bovine rate dropped to 11%.

These/.....

TABLE XVI

INCIDENCE OF HUMAN AND BOVINE BACILLI CAUSING
MENINGITIS IN SCOTLAND

Date	0-5 Years			6-15 Years			Over 15 years			Total		
	H	B	% B	H	B	% B	H	B	% B	H	B	% B
1907-37	178	72	28.8	71	19	21.1	30	6	16.6	281	99	26
1943-44	193	42	17.8	153	15	8.9	152	5	3.1	498	62	11

These results are summarised in Table XVI (on the opposite page) which also serves to prove another fact which is uniformly present in all typing experiments of this nature, namely that bovine infection is predominantly an infection of childhood, more milk generally being consumed at that age.

In England very much the same position exists as can be seen from the figures listed by Griffith (1937-38), derived from various authors, as being 24.6% for all ages. In the latest investigation in England (1943-45) the figure actually rises to 28% in a series of 254 cases examined.

A simultaneous investigation held in Wales (1943-45) provided a bovine incidence of 10% from a total of 69 cases examined.

In North Ireland, McMurray (1944) found 29% of 26 cases of tuberculous meningitis to be due to the bovine bacillus, while in a more recent investigation Reilly (1950) found only 3.3% of 245 cases of meningitis to be due to the bovine bacillus.

In Eire, Mushatt (1940) found 25% of 12 cases examined to be due to the bovine bacillus.

In Germany in 1932 Bruno Lange described the incidence of bovine meningitis to be 8.4% of 201 cases examined.

In Denmark the bovine incidence is described as being 23.4% by Jensen et al. (1940) and in France, Gervois (1937) found 7.4% of meningeal tuberculosis to be bovine in origin, while Gara (1942) working in the North of Italy (Milan) found a bovine incidence of 9.2% in 195 cases of extrapulmonary tuberculosis studied.

In Budapest, Vasarhelyi (1940) found the bovine incidence to be 12.8% of 48 cases examined, and in India, Ukil (1933) in typing 60 cases of extrapulmonary tuberculosis did not encounter a single case of bovine infection.

In Japan, Kimura and Kondo (1939) found the bovine incidence to be 0.98% in a series of 1,323 cases of extrapulmonary tuberculosis investigated.

In Amsterdam, Ruys (1939) reported a bovine incidence of 16.2% in 43 cases of tuberculous meningitis examined.

In Australia the incidence of bovine infection in tuberculous meningitis is 5% in a series of 80 cases reported in the literature (Penfold, 1924; Webster, 1932; Webster, 1941).

In America the following position exists. Park and Krumweide (1910) reported a bovine incidence of 15% in their series of meningitis cases examined in New York. Novick (1919), also working in New York, reported the incidence of bovine infection in 48 cases of meningitis as being 6.3%. In Boston, Gordon and Brown (1923) reported the bovine incidence as being 15.7% of 19 cases examined, while Herben and Asserson (1925), working in New York, reported a bovine incidence of 6% in a series of 47 cases examined. Apart from another investigation by Chang (1933), no other investigations of this nature have been reported since an extensive (and successful) campaign at eradication of bovine tuberculosis was instituted in that country in 1917 (Palmer, 1951).

Finlayson and Edson (1947), working in Dunedin, New Zealand, record 9 cases (19%) of bovine infection in 48 cases of extrapulmonary tuberculosis studied in that city. These figures include 8 cases of meningitis, of which 4 were of bovine origin.

The South African literature records the type determination of 243 strains of tubercle bacilli (Pub. S.A. Inst. Med. Res., 1932; Annual Rep. S.A. Inst. Med. Res., 1937; Harrington and Emerson, 1939; du Toit and Buchanan, 1942) 195 of which were derived from extrapulmonary sources. Only two were found to be bovine in origin.

As far as results of the present investigation are concerned, the incidence of bovine infection in non-

European cases of tuberculous meningitis is 2 in 184 or 1.1%, while the Europeans in this area have a rate of 0 in 16 or 0%.

That the incidence of bovine meningitis in the non-European population in this area must be very low and actually approximate to the figure (2 in 184, i.e. 1.1%) obtained in this series, can be deduced from the theoretical assumption that the true incidence is actually as high as 5%, under which circumstances the chance of not finding more than two bovine cases in a series of 184 cases of meningitis becomes 1 in 219. It is thus reasonable to infer that the true incidence is substantially less than 5%.

On the other hand the finding of no bovine strains in the series of 16 European cases investigated, i.e. 0%, does not allow such inferences regarding the true incidence of European bovine meningitis in this area to be made. Assuming the true incidence to be only 5%, (because of the small number of cases investigated) the chance of not encountering a bovine infection in the 16 cases examined is then actually as high as 44%. Even with a European bovine rate of 10% in this area, there is still an 18.5% chance of not striking a single bovine infection in the small series examined.

What then is the explanation of the difference existing between the incidence of bovine tuberculous meningitis in the Cape (1%) and that of the British Isles? Considering the position of the Coloureds (who form 82% of the cases), the tuberculous meningitis mortality rate existing amongst them in the Cape Province in 1947 (the most recent Annual Report of the Union Department of Health) is 17.9 per 100,000 of the population, as compared with 3.6 per 100,000 for England and Wales (Report of the Ministry of Health for the year ended 31st March, 1948), and 0.7 per 100,000 for the United States of America (Federal Security Agency National Office of Vital
Statistics/....

Statistics, 1950) over the same period of time.

This disparity is reflected in the differences existing between the respiratory death rates for tuberculosis amongst these groups of people: 208.2 per 100,000 for the Coloureds in the Cape Province (1947), as compared with 46.8 per 100,000 for England and Wales and 31 per 100,000 of the population in the U.S.A., for 1947.

Although the incidence of bovine infections in pulmonary tuberculosis in the Coloured population is not known, judging from results of other countries, it must be very low indeed and one can expect the majority of meningeal infections amongst the Coloureds, living as they do in crowded slum conditions, to be human in type, resulting from the tremendous reservoir of pulmonary tuberculosis existing in this section of the community.

Thus, while agreeing with Reilly (1950) that an argument like this can be open to certain objections, I maintain that the high meningeal death rate and the low bovine incidence existing amongst the Coloureds in the Cape is primarily due to the high respiratory death rate coupled with the fact (to be further elaborated) that very little milk is consumed, and they thus largely escape the danger of bovine infection.

To the Natives in the Cape Province, with meningeal and respiratory mortality rates of 5.27 per 100,000 and 126.2 per 100,000 respectively, exactly the same argument applies.

This state of affairs is well illustrated in the present series. One hundred and thirty-two of the cases occurred in the Cape Town Municipal area, which has a European population roughly equivalent to that of the non-European population, and the ratio of non-European to European cases in the latter area was 31:2.

It is, however, amongst Europeans in the Cape Province with mortality rates (pulmonary 21.8 per 100,000 and meningeal 3 per 100,000 for 1947) approximating those of/.....

of the British Isles, that the apparent discrepancy lies (no bovines in 16 cases), for this class of person consumes roughly a similar quantity of milk as do people in England and, as will be pointed out later, there is reason to believe that their milk supply is very little, if any, better as regards contamination with tubercle bacilli, than the milk consumed in Great Britain.

As will be mentioned later, contact with known cases of pulmonary tuberculosis was established in 11 of the 14 European cases investigated. The three failures, in children of 11, 15 and 18 years respectively could almost certainly be ascribed to the fact that at those ages, the field of possible contact is vastly greater than during the first years of life, making it proportionately difficult to trace possible contacts.

As mentioned previously, however, the findings in this series of 16 cases do not permit deductions as to the true incidence of bovine meningeal infections in Europeans to be made and the true picture must await further typing experiments on larger numbers of cases.

In an attempt to verify the claim that the human case of pulmonary tuberculosis is by far the most important epidemiological factor in tuberculous meningitis in this area, an attempt was made to investigate the environment of the 200 cases examined bacteriologically, with the object of tracing each meningeal infection to its ultimate source.

With this in mind it was decided to investigate every case as follows:-

- (1) Examine, and if possible submit all people normally coming into contact with the patient to an X-ray examination. A particular person was considered the source of the patient's tuberculosis when he or she presented definite clinical, or preferably, radiological evidence of pulmonary tuberculosis; and also when it had been established that such a person had been in contact with the patient for some period of time.
- (2) Investigate the milk supply of the patient where necessary.

Credit for most of the data presented in this section must go to people other than myself - to the staffs of the various Municipal and Divisional Council Tuberculosis Clinics, doctors at the City Hospital for Infectious Diseases, and most important of all, the health visitors attached to the various clinics, to whom the task of getting the various people to attend and submit to an X-ray examination was largely entrusted.

It soon became obvious that this was a most difficult task for a large number of reasons, not the least important of which was the apathy encountered amongst patient's relatives. Apathy was present to such a degree that many parents never appeared at the particular hospital to which their child had been admitted, some even failing to claim the body after death. In some instances (especially country cases) this could be ascribed to lack of money required for the necessary transport.

It was also apathy and a woeful lack of any understanding of the disease which prevented many parents and close relatives from attending the various clinics for examination, even upon repeated requests to do so.

Another factor was the hopeless state of overcrowding in which most of these people live - two families often sharing one room, or three or more families sharing one house. It was practically impossible to get all the occupants of one house, or even one room, sufficiently interested to come up for an examination.

Other factors operating were the frequency with which these people changed their addresses, gave wrong addresses or addresses which subsequently could not be traced. Finally, of course, there was the very real fear existing amongst possible contacts of being pronounced tuberculous and losing employment as a result of such a diagnosis, especially as chances of hospitalisation are very slight for most of them.

Through lack of facilities country cases were hardly
ever/....

ever investigated.

As regards the other possible source of tuberculosis - milk - it was found that, especially amongst the non-Europeans, if any milk was consumed it was usually bought casually at neighbouring dairies, a regular delivery of milk being unheard of.

These dairies in their turn buy milk in bulk from various and variable sources, and under these conditions I agree with Munro and Scott (1936) that it is practically impossible to trace a human bovine infection to a particular cow.

It takes at least 6 weeks (usually longer) for the isolation and typing of a tubercle bacillus and more than that period of time for the disease to become manifest in the patient. These facts, taken in conjunction with the statement that a cow secreting bacilli of this nature is almost certainly a poor milker, explain the difficulty encountered in actually incriminating any particular cow for a case of human bovine infection as the astute dairyman has usually disposed of the animal by the time investigations are instituted. Most investigators have thus simply confined themselves to the question whether or not the patient was in the habit of consuming raw cow's milk.

These are the reasons why the one bovine case (a Coloured child) occurring in Cape Town could not be traced to a tuberculous cow, although it was established that the child did consume a certain amount of unboiled cow's milk and that there were no cases of pulmonary tuberculosis amongst his immediate family. An attempt was made to investigate the other bovine case (a Coloured farm labourer's child on a farm in the Piketberg district) but owing to various difficulties, not the least important of which was the attitude of the particular farmer concerned, this was also unsuccessful. It was, however, established that the child did regularly consume a fair amount of raw farm milk.

Various/.....

Various details regarding the remaining 198 patients, including the results of the contact investigations, are listed in Table XVII at the end of this Chapter.

The comment "Not investigated" behind the names of 36.9% of these patients implies that either none of the possible contacts could be investigated, or, as more frequently happened, only a minority of the people living with the child were examined and found to be non-tuberculous. This figure compares very badly with a series of 182 cases investigated in England (1943-45) in which no information (in the above sense) was obtained in only 13.7% of cases.

Summarising these results it can be seen that out of a total of 127 cases due to the human type of bacillus investigated, contact with a case of respiratory tuberculosis was established in 112 instances (88.1%), 78.6% for European cases investigated, 89.8% and 100% for Coloured and Native cases investigated respectively, and 0% for one Asiatic case investigated.

Various authors have reported varying degrees of success regarding investigations of this nature and their results are listed in Table XVIII.

TABLE XVIII.

CONTACT INVESTIGATIONS IN CASES OF MENINGITIS DUE TO
THE HUMAN TUBERCLE BACILLUS

Author	No. of Cases Investigated	% Contacts
Meyer (1915)	105	27
Herben and Asserson (1925)	135	56
Greengard (1928)	54	24
Blau (1929)	110	24
Rosenblum and Makler (1932)	101	19
Investigation England (1943-45)	157	39.5
Investigation Wales (1943-45)	57	22.8
McMurray (1944)	10	80
Lincoln (1947)	167	57

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McMurray (1944)	10	80
Lincoln (1947)	167	57

In addition Munro and Scott (1936) and Reilly (1950) report that most of their cases due to the human bacillus could be traced to a case of human pulmonary tuberculosis.

The relationship of the contact to the patient in this series of cases is set out in Table XIX.

TABLE XIX.

RELATION OF CONTACT TO PATIENT

Relation to Patient	Number of Cases
Lodger	38
Mother	18
Father	15
Uncle	14
Aunt	6
Brother	4
Sister	4
Maid	3
Friend	2
Grandfather	2
Grandmother	1
Greatgrandfather	1
Greatgrandmother	1
Cousin	1
Neighbour	1
Landlord	1

Worthy of note is the fact that a tuberculous non-European maid was the source of infection of three European children with tuberculous meningitis.

These results differ from those of other investigators (Blacklock, 1947; Lincoln, 1947) who incriminate close relatives (mothers and fathers) more frequently than unrelated people in the same house (lodgers), and is possibly a reflection on the state of overcrowding existing in the homes of most of the patients.

An interesting point brought out by this investigation, as also the English investigation (1943-45), is the decline in the contact rate with increase in age (Table XX).

Table XX/.....

TABLE XX

AGE - CONTACT RELATIONSHIP

Age	No. of Cases Investigated	% of Contacts
0-5	96	100
5-10	14	50
10-15	9	66
Above 15	8	50

Thus between the ages of 0 and 5 years the contact rate for investigated cases is 100%, between the ages of 5 and 10 years the contact rate falls off to 50%, rising to 66.6% for cases between 10 and 15 years, and for cases above 15 years, the contact rate for investigated cases again falls to 50%. This presumably is due to the widening of the field of possible contact with increase of age.

Cattle form the great, and practically only, reservoir of bovine infection and contaminated milk acts as the vehicle for transmission to humans in the large majority of cases, although (as has been mentioned) air borne infection is also known to occur.

Milk being the vehicle, infection with the bovine type of bacillus is mainly through the alimentary system and accounts for the greater proportion of cases of cervical lymph node and abdominal tuberculosis in the various Scottish and English investigations.

For alimentary bovine infection to occur in a community, two requirements must be met, namely: (1) The milk supply must contain viable tubercle bacilli; and (2) the population must be exposed to the infection by drinking the contaminated milk. With regard to the first point, the position existing in Cape Town is not definitely known as no generalised tuberculin testing scheme has been undertaken amongst the dairy cows, and abattoir/.....

abattoir figures are unreliable for the simple reason that diseased cows are usually not submitted to the Cape Town abattoir, but sent to the small peri-urban abattoirs where control is less strict (Cooper, 1952).

The only extensive tuberculin survey carried out in this country amongst dairy cattle is the investigation attempted in Durban in 1930 (Green, 1933). 39.9% of dairy cows in that area were found to be positive reactors. The very high incidence of 6.4% of these cows were found to have tuberculous disease affecting the udder, and I agree with de Kock (1934) and Sampson (1934) in concluding that data such as this, from an urban area like Durban, seem to indicate that the incidence of tuberculosis in dairy cattle in Cape Town, or for that matter any city in the Union, is in no way different from that existing in various European countries.

What, however, is known about the milk supply of Cape Town is that Horwitz (1944) reported viable tubercle bacilli in 3.4% of herd samples supplying milk to the City. The figure for 1950-51 (Horwitz, 1951) is 2.7%. It must be stressed that these figures apply to herd samples and once the milk is bulked, the figure is correspondingly greater. In Johannesburg, Pullinger (1942) however reported the figure to be 2.5% of bulked samples.

In Great Britain (Wilson, 1942; Francis, 1947; Allen, 1949) about 40% of cows slaughtered in public abattoirs are tuberculous; about 0.5-1.0% or more of these cows (Stamp, 1943) have the disease affecting the udder (and may thus be expected to secrete tubercle bacilli in their milk), and 7.5% of churn (presumably bulked) samples contain viable tubercle bacilli (Francis, 1947).

The position in Scotland at the moment is more favourable than that existing in Wales or England with

41% of dairy herds as compared with 30.5% and 15%, for Wales and England respectively, attested free from tuberculous disease (Kirkpatrick, 1951), figures which are accurately reflected in the incidence of bovine meningeal infections in these countries in the latest investigations carried out.

In New Zealand approximately 10% of dairy cattle are tuberculous (Finlayson and Edson, 1947).

In America, where a bovine tuberculosis eradication scheme has been in progress since 1917 (Public Health Reports, 1946, 61, 1315), the incidence of tuberculous cows is now less than 0.3% and human bovine tuberculous infection is no problem at all. (Editorial, Post Graduate Med. J., 1949, 25, 463; Palmer, 1951)

In North Ireland the position is very similar to that in Great Britain - 40% of cows are tuberculous and 7.5% of churn samples contain viable tubercle bacilli (Reilly, 1950).

In 1949 the incidence of positive tuberculin reactors amongst dairy cattle in Japan was only 0.48% (Crawford, 1952) and in India the incidence, while not definitely known, is generally regarded as being insignificant, and no case of tuberculous mastitis has ever been recorded in that country (Crawford, 1952).

Tuberculous milk can, of course, be rendered harmless by subjecting it to some form of heat treatment, i.e. the various forms of pasteurisation or boiling (Wilson, 1942) and at the moment about 60% of milk sold in Cape Town is pasteurised (Horwitz, 1951). In England prior to 1923 effective pasteurisation was negligible, but by 1938, 50% of milk consumed in county boroughs and 98% of milk consumed in London was pasteurised (Kirkpatrick, 1951).

Owing to lack of facilities, milk sold in country
places/.....

places is hardly ever pasteurised and neither is the infective agent diluted by the process of bulking which is carried out in the larger centres.

Human infection with the bovine tubercle bacillus would thus be expected to be higher in country towns than in large cities and this is well illustrated by Letham (1946) who, taking the death rate from abdominal tuberculosis in young children as an index of the incidence of bovine infection, gives a table to show the effect of pasteurisation in reducing the incidence of this infection. Table XXI.

TABLE XXI.

DEATH RATES FROM ABDOMINAL TUBERCULOSIS PER MILLION CHILDREN UNDER 5 YEARS OF AGE IN EACH AREA.

	1921	1930	1938	1944
London Administrative County	136	24	12	6
Combined County Boroughs	437	157	63	35
Combined Urban Districts	366	134	77	42
Combined Rural Districts	252	92	63	60

I may add that one of my two cases of bovine infection occurred in a Coloured farm child.

Another factor in this difference in the incidence of bovine infection in urban and rural inhabitants is an immunological one. Infection with the human type of bacillus must be less common in the country than in the towns, and the rural dweller, therefore, escapes this type of infection; he consequently develops no tuberculo-immunity, and is thus more liable than the town inhabitant to develop bovine infection (Blacklock, 1947). Indeed it has been suggested (Sampson, 1934) that the presence of bovine bacilli in milk may serve as an immunising agent for the human subject. I could not disagree more strongly with such a doctrine, for although there is a close antigenic relationship

between/.....

tuberculosis (that of consuming the contaminated milk), the highest incidence of bovine infection is found in early life and is probably due to the greater consumption of milk at that age. There are thus greater opportunities for infection should the milk contain viable tubercle bacilli. As the mucosa of the intestine and the nasopharynx (Blacklock, 1947) are less favourable sites for attack than the lung parenchyma, by tubercle bacilli which grow slowly, some co-incident lesion may probably allow the bacilli to invade these mucosae. The more frequent occurrence of acute catarrhal conditions at these sites in children, as compared with adults, may provide the *locus minoris resistentia* in which the tubercle bacilli may gain a foothold. This may partly account for the greater frequency of tuberculosis of the cervical lymph nodes and the abdomen in children than in adults. Further, the possibility of acquired resistance due to an arrested infection of the lungs may also determine the relative infrequency of alimentary tuberculosis in the adult as compared with the child.

The above, of course, only applies to people with a reasonable standard of living who can afford the expense of buying milk regularly. Amongst the non-European population of Cape Town, with whom, due to their sheer weight of numbers in this investigation, we are mainly concerned, drinking a glass of milk is a habit practically never indulged in at home. The usual practice is either to forgo it altogether, or to use it sparingly in tea or coffee. If milk is occasionally bought, it is usually boiled at once to prevent it going sour and more often than not condensed milk is substituted because it can be kept longer without going off. The practice of having a regular delivery of milk is unheard of.

Most infants are breast fed and, if necessary, are
supplied/.....

supplied with powdered milk at the various municipal clinics, and some of the older children receive pasteurised milk at school.

Thus as a result of drinking very little milk, the non-European population is hardly exposed to the hazard of bovine tuberculosis.

The European population on the other hand consumes a large amount of milk and one would deduce from facts quoted, that they at least should have a bovine infection rate roughly similar to that existing in the British Isles. My result, of no bovine infections in 16 cases, is, however, not statistically significant.

Table XXII correlates the incidence of human bovine infections in various countries with the inhabitants' milk-drinking habits and the incidence of tuberculosis present in the dairy cattle.

TABLE XXII.
CORRELATION OF THE MILK DRINKING HABITS OF VARIOUS PEOPLES
WITH THE BOVINE TUBERCULOSIS RATE IN
CATTLE AND LAB.

Country.	Tuberculous Infection in Dairy Cattle.	Heat treatment of Milk.	Human Consumption of Milk.	Human Bovine Tuberculosis Rate.
Japan	Negligible (0.48%).	Habitually boil milk.	Average	Negligible
India	Low	Habitually boil milk.	Average	Negligible
America	Negligible	Variable.	Abundant	Non-existent
England and Scotland	Heavy (40%)	Variable.	Abundant	High
North Ireland	Heavy (40%)	90% Pasteurised.	Abundant	Low
Western Province (Non-European)	Heavy	Variable.	Minimal	Negligible

As regards respiratory infection with the bovine tubercle bacillus, Munro (1929) was the first to describe a case of human pulmonary tuberculosis due to the bovine bacillus. This discovery, of course, at once raised the question of the pathogenesis of the lesion, and/.....

and two possibilities existed; namely a spread to the lung from the primary alimentary complex, or an air borne (droplet) infection as commonly occurs in the case of the human bacillus. That the latter is the usual mode of infection has since been decisively proved by Blacklock (1932), and further proof of air borne infection has been furnished by Jensen et al. (1940), Cutbill and Lynn (1944) and by Sigurdsson (1945).

The disease being airborne, the highest incidence of human bovine pulmonary tuberculosis should be found amongst people coming into direct contact with cattle, and this has definitely been proved to be the case; Cutbill and Lynn (1944) give the incidence of human bovine phthisis in cattle contact and non-cattle contact groups as follows: 97 cattle contacts, 16.4% bovine; 2004 non-cattle contacts, bovine incidence 1.6%. The cattle contacts are usually farmers or farm labourers.

The incidence of pulmonary bovine tuberculosis is, however, relatively small accounting for 5.7% of cases in Scotland and 1.9% of cases in England, Wales and Eire, in the collected figures for all ages reported by Griffith and Munro (1944).

Cumming (1935) typed 320 strains of tubercle bacilli derived from cases of pulmonary tuberculosis in Eire and found no case due to the bovine organism; and more recently, Kearney, Farrelly and Cronin (1949) typed another 500 cases in the same country, also failing to find a single case of bovine infection.

Sigurdsson (1945) describes the amazing incidence of 40.6% of bovine infections in a study of 165 rural cases of pulmonary tuberculosis in Denmark. This, however, becomes less surprising when it is realised that during the cold Danish winters cattle are often allowed into the immediate precincts of the houses of farmers

(de Kock, 1951) and a very close relationship thus exists between cattle and man, the former (if tuberculous) disseminating tubercle bacilli by droplet infection in exactly the same way as a case of human pulmonary tuberculosis would do it, and Sigurdsson actually traced 94% of his bovine cases to "strongly tuberculous cattle".

In the British Isles the human bacillus is responsible for only a relatively small percentage of primary alimentary infections, the figures for Scotland collected from 1914-1944 (Mitchell, 1914b; Wang, 1916-17; Blacklock, 1932; National Investigation Scotland, 1943-44) showed total human infections of 22.1% (77.9% bovine). The latest English figures (1943-45 survey) gives the human tubercle bacillus incidence in abdominal infections to be 35.3% (64.7% bovine). To account for these primary alimentary infections by the human type of bacillus, the bacillus must have been swallowed in food contaminated by droplet infection (Blacklock, 1947).

As judged by the following facts, the incidence of primary abdominal infections in this area must be very low, for firstly, in a series of 90 post mortem examinations done as part of this investigation, only one primary abdominal infection was encountered. As will be discussed later on page 113, these results differ markedly from similar series in the British Isles and tend to confirm the results of the typing experiments performed on tubercle bacilli derived from these particular cases.

Secondly, in a study of the records of the Department of Pathology of the University of Cape Town (which drains an 800 bedded general hospital for material), only 10 cases of abdominal tuberculosis (caseous mesenteric lymph nodes, no tuberculous hilar lymph nodes and possibly only haematogenous pulmonary tuberculosis)

were/.....

were encountered in a consecutive series of 2,365 post mortem examinations performed during the years January, 1945 to September, 1950. Only 3 of the 10 patients were Europeans but, as the causal organisms were not typed, the bovine incidence (with its implications) is not known.

Of the cases of primary tuberculosis of the cervical lymph nodes investigated up to 1944 in Scotland (Mitchell, 1914a; 1914b; Griffith, 1916; 1929; Wang, 1916-17; Blacklock, 1932; Griffith and Smith, 1935; Scottish National Investigation, 1943-44) the rate of infection with the human type was 27.8% (72.2% bovine).

The latest English figures (1943-45) gives the percentage human infection of cervical lymph nodes to be 42.5% (57.5% bovine).

The position regarding the types of infection in cervical lymph nodes existing in the Cape has never been reported, but in an investigation in progress at present, tubercle bacilli isolated from the cervical lymph nodes of 30 non-European patients have all been typically human in type. This remarkable result is explained on exactly the same lines as those given for meningeal infections, namely a very high incidence of pulmonary tuberculosis coupled with a minimal consumption of raw cow's milk by the Non-European population. This tremendous reservoir of pulmonary tuberculosis results in widespread dissemination of human bacilli which, in the overcrowded conditions existing amongst the non-Europeans, accounts for the high incidence of primary thoracic infections by inhalation of infected droplets, and the high incidence of human tubercle bacilli infections in cervical adenitis from the ingestion of bacilli deposited in droplets on food, floors, walls and furniture.

TABLE XVII.

Results of Contact Investigation.Coloured Patients.Pulmonary Tuberculosis Contacts.

Name.	Address.	Age.	Sex.	Pulmonary Tuberculosis Contacts.			
				Name.	Relation to Patient	Notified as Pul. T.B. on.	Remarks.
Henry Booysen	75 Hanover Street, Cape Town	4 mths.	M.	Jacob Gedult	Uncle	31. 8.50.	Same Address.
Rosetta Samuels	40 Overton Rd., Zone B., C Town, Athlone	4 mths.	F.	Eileen Samuels	Mother	19. 3.45.	
Rachel Alexander	38th Str., Off Belrine Rd., Elsies River.	4 mths.	F.	Henry Lalloo	Step- brother	22. 7.46.	
Janap Sokaldien	"Break of Day", Harmony Rd., Hazendel Est., Athlone.	5 mths.	F.	Gadija Galant	Lodger	22. 8.37.	Same Address.
Nabia Galant	"Break of Day", Harmony Rd., Hazendel Est., Athlone	5 mths.	F.	Gadija Galant	Mother	22. 8.37.	
Baby Davies	7 Faith Str., Claremont	5 mths.	F.	Susan Daniels	Aunt	7. 7.50.	Same Address.
Moses Petersen	c/o Klyn & Strandfontein Rd., Wynberg.	5 mths.	M.	Jack Paulse	Lodger	2. 7.49.	" "
Ebrahim Seeman	Off Johnson Road, Athlone.	6 mths.	M.	Maggie Telen	Aunt	3. 7.49.	" "
Audrey Lötter	65 Canterbury Flats, Cape Town.	6 mths.	F.	Sarah Lötter	Mother	4.10.50.	
Baby Sartz	Church Street, Somerset West.	6 mths.	F.	-	-	-	Not Investigated.
Brian Daniel	13 Glyndale Str., Cape Town	6 mths.	M.	Lena Fortuin	Lodger	12. 7.47.	Same Address.
Omar Hendricks	Hampton Estate, Crawford.	6 mths.	M.	Juria Abrahams) Charlie ")	Neigh- bours.	(21. 7.50 (29. 8.50	
Janet Raubenheimer	"Moravia", Derby Rd., Lens- downe.	7 mths.	F.	Rachael Rauben- heimer.	Mother	29. 1.51	

TABLE XVII. (continued).

Coloured Patients

Name.	Address.	Age.	Sex.	Pulmonary Tuberculosis Contacts.			
				Name.	Relation to Patient	Notified as Pul. T.B. on.	Remarks.
Joyce Kester	93 Albert Rd., Woodstock.	7 mths.	F.	Sam Martin	Father	8. 2.51.	
Eva de Bruyn	End of Glenboig Rd., Parow.	7 mths.	F.	Frank de Bruyn	Father	15. 3.50.	
Maurita Cupido	53 Appledene Rd., Zone B., Silvertown, Athlone.	7 mths.	F.	Sam Cupido	Uncle		Died of T.B. Dec. 1950. Same Address
Michael Clarke	No. 1 Fifth Str., Strand	7 mths.	M.	Maggie Telan	Aunt	3. 7.49.	Same Address.
Valerie Elliot	12th Ave., (off St. George's Drive) Retreat.	8 mths.	F.	Anna Elliot	Mother	2. 3.51.	
Carol Moses	297 Bloemhof Flats, Block B. McKenzie St., Cape Town	8½ mths.	F.	Olga Moses	Mother	20. 9.50	
John Manuel	8th Str., 6th Avenue, Kensington.	9 mths.	M.	Jack Pope	Lodger	12. 7.43	Same Address.
Anthony Moses	82 Dorp Street, Cape Town.	9 mths.	M.	Richard Salie	Lodger	26.11.46	Same Address.
Susan Chippendale	6 Rock Road, Black River.	9 mths.	F.	David Julius) Michael Coetzee)	Uncle Lodger	23. 2.50) 16. 8.48)	Same Address. Same Address.
Emily Scott	16 Dove Str., Salt River.	9 mths.	F.	John Scott	Uncle	29.11.47	Same Address.
Dawn Knight	6th Str. off 2nd Ave., Kensington.	9 mths.	F.	Magdalena Knight	Sister	7.12.48.	
Robert Kleinsmidt	Ruhob No.13 16th Ave., Crawford.	9 mths.	M.	Audrey Dantu (Abrahams)	Lodger	14. 8.46.	Same Address.
Rosaline de Villiers	103 Acre Rd., Goodwood	10 mths.	F.	Willem de Villiers	Father	19. 1.50	
Isaac v.d. Vent	35 1st Ave., Bokmakierie	10 mths.	M.	John v.d. Vent	Father	28. 5.51.	

TABLE XVII. (continued).

Coloured Patients.

Pulmonary Tuberculosis Contact.

Name.	Address.	Age.	Sex.	Pulmonary Tuberculosis Contact.			
				Name.	Relation to Patient.	Notified as Pul. T.B. on	Remarks.
Andrew Gamba.	115 Cambridge Str., Elsie's River.	11 mths.	M.	-	-		Not investigated.
John Clingen.	23 Tennison Str., Salt River	11 mths.	M.	Joseph Isaacs	Lodger	7. 9.49.	Same Address.
Gamat Kassiem.	Kiavia Strothallon Rd., Black River.	1 yr.	M.	-	-		Not Investigated.
Wilfred George.	Off Baths Rd. Wynberg.	1 yr.	M.	(Mavis Smith (Maud Smith	(Auntie (Cousin	20. 8.43) 9. 1.51)	Same Address. Same Address.
Dan Stephens.	Pinedeane Rd., Tiervlei.	1 yr.	M.	-	-		Not Investigated.
Francis Pirie.	27 London Rd. Salt River.	1 yr.	F.	Richard Dirk.	Great Grandfather.	23. 8.50	Same Address.
Joey Martin.	13th Av., Windermere.	1 yr.	F.	Peter Martin	Father		Screened City Hosp Active T.B.
Rhoda Adams.	Fernwood Estate, Aberdeen Cott., Claremont.	1 yr.	F.	Gerald Adams	Uncle		D. 16/5/50 of Pul. T.B.
John Maasdorp.	Block 2, No. 9 Q. Town, Athlone.	1 yr.	M.	Susan Maasdorp	Auntie	20.10.48	Same Address.
Galima Majiet.	36 c/o Pancras and Thornton Rds., Cleemoor. Athlone.	1 $\frac{1}{2}$ yr.	F.	Navaine Majiet	Mother	29.6.50	
Jessie Makelila.	c/o Mr. A.A. Bray, Grassmere Str., Athlone.	1 $\frac{1}{2}$ yr.	F.	-	-		Not investigated.
Jan Maart.	Tulbagh.	1 $\frac{1}{2}$ yr.	M.	-	-		Not Investigated.
Jennifer Hendrikse.	1 Huxley Ave., Athlone.	1 yr. 2 mths.	F.	Millie Krotz	Lodger	14. 3.50.	Same Address.
Erenda Rudolf.	Mossel Str., Parow.	13 yr. 12	F.	-	-		Not Investigated.

TABLE XVII. (continued.)

Coloured Patients.

Pulmonary Tuberculosis Contacts.

Name.	Address.	Age.	Sex.	Pulmonary Tuberculosis Contacts.			
				Name.	Relation to Patient	Notified as Pul. T.B. on.	Remarks.
Elizabeth Robertson (Langley)	3rd Street, 9th Avenue, Windsmere.	14 12 yrs.	F.	Doris Langley.	Mother.		Screened City Hosp. Active T.B.
Eidronise Shadrick	No. 2 Jays Villa, Upper Pep- per Str., Cape Town.	1½ yrs.	F.	Bobby Naidoo.	Lodger.	19. 1.50.	Same Address.
Merian Kahn	c/o Jamal's Shop, Thornton Rd., Gleemoor, Athlone.	1½ yrs.	F.	Allie Kahn.	Uncle.	6. 2.47.	Lives next door.
Marida Hamet.	47, Rodger Street, Cape Town.	1½ yrs.	F.	Ebrahim Kedar.	Lodger.	19. 7.50.	Same Address.
Petronella Mamatu.	Rylands Estate, Klipfontein Rd., Athlone.	1½ yrs.	F.	-	-	-	Not Investigated.
Marida Hammond.	Cassels Vlei Rd., Bellville South.	1½ yrs.	F.	-	-	-	Not Investigated.
Melvyn Raymond.	Vauxhaul, off de Waal Drive, Cape Town.	1½ yrs.	M.	Regina Raymond.	Mother.	12. 1.49.	
Henriette Cloete.	Teroma, Upper Park Rd., Walmer Est. Woodstock.	1½ yrs.	F.	Abraham Cloete.	Father.	10. 7.51.	
Stanley Lakay	Lily Villa, 5 Mowbray Rd., Maitland.	1½ yrs.	M.	Isabella Mathews	Lodger	2. 3.49.	Same Address.
Serida Solomons.	c/o 3rd Ave. Klipfontein Rd., Elsies River.	1½ yrs.	F.	David Solomons.	Uncle.	19. 7.50.	Same Address.
Roseline Lakay.	24, Strand Roos Close, O. Town, Athlone.	1½ yrs.	F.	-	-	-	Not Investigated.
Wilfred Davids.	3rd. Avenue, Grassy Park.	1½ yrs.	M.	-	-	-	Not Investigated.
Grace Isaacs.	6th. Avenue, Grassy Park.	1½ yrs.	F.	Susan Isaacs.	Mother.		Died on 12.4.50. of Pulmonary T.B.

TABLE XVII. (continued.)

Coloured Patients.

Name.	Address.	Age.	Sex.	Pulmonary Tuberculosis Contact.			
				Name.	Relation to Patient	Notified as Pul. T.B. on.	Remarks.
Zonia Williams.	5 Cross Bulward, O. Town, Athlone.	1½ yrs.	F.	Willem Stout.	Uncle.	25. 6.47.	Same Address.
Brain Mathews.	6 van der Lear Str. Cape Town.	1½ yrs.	M.	Joseph Mathews.	Uncle.	5. 7.44.	Same Address.
Valerie Lawrence.	7 Kannabast Close, O. Town, Athlone.	1½ yrs.	F.	Dorothy Williams.	Lodger.	24. 4.48.	Same Address.
Doreen Cornelissen.	Moor Street, Pearl.	1½ yrs.	F.	-	-	-	Not Investigated.
Carol Robson.	c/o 4th Ave. and 5th Str. Kensington.	1½ yrs.	F.	Pieter Zaal.	Lodger.	22. 7.48.	Same Address.
Sarah Hammersley.	No. 8 off Myburg Road, Diep River.	1½ yrs.	F.	-	-	-	Not Investigated.
Pamela Ndzana.	3rd Ave. Welcome Estate, Athlone.	1½ yrs.	F.	-	-	-	Not Investigated.
Geronessa Sellers.	55 Pepper Str., Cape Town.	12 yrs.	F.	Rachmat Losper (Edwards)	Lodger.	8. 3.33.	Same Address.
Moosa Job.	16th Str. 6th Ave., Kensington	2 yrs.	M.	Alice Stafford.	Lodger.	15. 2.50.	Same Address.
Gamat Hendricks.	16 Vrede Str., Cape Town.	2 yrs.	M.	Johanna Barron	Lodger.	6. 1.47.	Same Address.
Solaymen Ismael.	Witkyk, 3rd Ave., Kensington.	2 yrs.	M.	Anthony King.	Lodger.	1.10.50.	Same Address.
Harold Petersen.	Abdullah's Cottages, Main Rd., Constantia.	2 yrs.	M.	-	-	-	Not Investigated.
Paul Adams.	11 de Villiers Lane, Cape Town.	2 yrs.	M.	Tommy Adams.	Brother.	6.12.43.	
				Paul Adams.	Brother.	13. 4.37.	

TABLE XVII (continued)

Coloured Patients.

Name	Address.	Age.	Sex.	Pulmonary Tuberculosis Contacts.			
				Name.	Relation to Patient.	Notified as Pul. T.B. on.	Remarks.
Salena Toffie	2nd Ave. Belgrave, Athlone.	2 yrs.	F.	Sallie Hattingh.	Uncle.	8. 2.51	Same Address.
Rosie Cornelius	287 Coronation Rd., Maitland	2 yrs.	F.	Sarah Cornelius.	Mother.	4.10.50	
Sophia Adams.	Townsend Str., Goodwood.	2 yrs.	F.	-	-	-	Not Investigated.
Jean Appels.	41 Haigh Ave., Graden Village Maitland.	2 yrs.	F.	Jane Appels.	Cousin.	4.11.45	Same Address.
Anne Smith.	6th Ave., Bokmakierie.	2 yrs.	F.	Johanna Smith.	Sister.	31. 4.48	
Everard Fortuin	Bath Road, Caledon.	2 yrs.	F.	Chrisjan Nel.	Lodger.		On waiting list for Nelspoort Sanatorium.
Amaline Simons	29 Coronation Road, Woodstock.	2 yrs.	F.	John Summers.	Grandfather	27. 4.50	Same Address.
William Otto	Park Street, Porterville.	2 yrs.	M.	-	-	-	Not Investigated.
Magdalena Mathews	4 van der Meulen Str., Cape Town.	2 yrs.	F.	Shadrick Daniels.	Lodger.	22. 1.50	Same Address.
Jennifer Rhode.	202 Andringa Str., Stellenbosch.	2 yrs.	F.	Willie Adams.	Lodger.		Notified through Health Centre, Stellenbosch.
John Jacobs	Fairway, Lake Rd., Grassy Park.	2 yrs.	M.	Samuel Jacobs.	Brother		Died of T.B. in City Hospital.
John Smith.	The Nook, Hope Street, Claremont.	2 yrs.	M.	Corrie Hendricks.	Lodger.	30. 5.45.	Same Address.
John Edwards	Stellenbosch.	2 yrs.	M.	-	-	-	Not Investigated.
Lorraine Felix	27, 36th Ave., Elsie's River.	2 yrs.	F.	Gerald Felix.	Brother.	13. 5.50	

TABLE XVII (continued)

Coloured Patients.

Name	Address.	Age.	Sex.	Pulmonary Tuberculosis Contacts.			
				Name.	Relation to Patient.	Notified as Pul. T.B. on.	Remarks.
Nellie Orlain	51 Garden Village, Somerset West.	2½ yrs.	F.	-	-	-	Not Investigated.
Leonard van Hagt.	Belgrave Est. Athlone.	3 yrs.	M.	John van Hagt.	Father.	31.10.46	Not Investigated.
George Kock.	c/o Y. van der Merwe, Vrolikheid, Tulbagh.	3 yrs.	M.	-	-	-	
Joan Ambril.	73 Sixth Str., Elsie's River.	3 yrs.	F.	-	-	-	Not Investigated.
Margaret Jordaan.	40, 11th Ave., Elsie's River.	3 yrs.	F.	Mr. Jordaan.	Father.	-	Screened City Hosp. Active T.B.
John Mentoor.	123 Weiners Str., Vasco.	3 yrs.	M.	Caroline Mentoor.	Mother.	4. 1.51.	Notified through Somerset West Municipality. Same Address.
Tommy Petersen.	Grodon Str., Somerset West.	3 yrs.	M.	Edward Rhooode	Lodger.	-	
Julia Arries.	9 van der Leer Str., Cape Town.	3 yrs.	F.	Joseph Smith.	Lodger.	2.12.48.	Same Address.
Magadene Adams	94 Aspeling Street, Cape Town.	3 yrs.	M.	-	-	-	Not Investigated.
Miriam Masik.	Bibila House, 4th Ave., Athlone.	3½ yrs.	F.	Africa Masik.	Uncle.	2.11.50.	Same Address.
Miriam Jacobs.	96, 8th Ave., Windermere.	3½ yrs.	F.	Broadway Masik.	Cousin	24.11.50	Same Address.
Pieter Cloete.	70 Place of Safety, Q.Town Athlone.	4 yrs.	M.	-	-	-	Not Investigated.
Sylvia Fortuin	C.S, Matroosfontein.	4 yrs.	F.	Jacob Abrahams.	Lodger.	2. 7.49.	Same Address.
Andries Plaatjies	c/o Modderdam Rd., and 12th Ave., Bellville South.	4 yrs.	M.	-	-	-	Not Investigated.

TABLE XVII (continued).

Coloured Patients.

Name.	Address.	Age.	Sex.	Pulmonary Tuberculosis Contacts.			
				Name.	Relation to Patient.	Notified as Pul. T.B. on	Remarks.
Harriet October.	12th Ave., Bellville South.	5½ yrs.	F.	-	-	-	No Contact.
Moosa Haasdorp.	Klipfontein Rd., Athlone.	6 yrs.	F.	-	-	-	Not investigated.
Valerie Petersen.	Forrester Rd., Werste River.	6 yrs.	F.	-	-	-	Not Investigated.
Grace Gordon.	Jasmine, Galway Rd. off Roscommon Rd. Heathfield	6 yrs.	F.	Dorothy Gordon.	Aunt.	28. 1.44.	Same Address.
Nora Phillips.	No. 13, 5th Ave., Bokmaki-rie.	6 yrs.	F.	Annie Phillips.	Mother.	-	Died 14.5.1949 of Pulmonary T.B.
Johanna Scholtz.	24th Ave., off Klipfontein Rd., Elsie's River.	6 yrs.	F.	-	-	-	Not Investigated.
Johannes Smit.	c/o Rev. Loodtz, Soetweide Str., Stellenbosch.	6 yrs.	M.	-	-	-	No Contact.
William Woolfe.	c/o Mr. Aandriagh, de Bron Brackenfel.	6 yrs.	M.	-	-	-	Not Investigated.
Anita Christians.	22 de Gama Str., Brooklyn.	6 yrs.	F.	-	-	-	No Contact.
Ephraim Davids.	18 Frene Str., Cape Town.	6 yrs.	M.	Armine Kleinsmidt.	Lodger.	25. 3.49.	Same Address.
Asa Samsodien.	6 A Combrink Str., Cape Town.	7 yrs.	F.	-	-	-	No Contact.
Fatima Maxwell.	58 Jordaan Str., Cape Town.	7 yrs.	F.	Alie Maxwell	Father.	5. 4.51.	-
Anna Bougat.	c/o Mr. le Roux, Burgundy Franchhoek.	7 yrs.	F.	-	-	-	Not Investigated.
Eileen Olivier.	52 Beattie Ave., Garden Vil- lage, Maitland.	7 yrs.	F.	-	-	-	No Contact.

TABLE XVII (continued)

Coloured Patients.

Pulmonary Tuberculosis Contacts.

Name.	Address.	Age.	Sex.	Pulmonary Tuberculosis Contacts.			
				Name.	Relation to Patient	Notified as Pul. T.B. on.	Remarks.
Lavona Adams.	Spes Bona, Lower Deuchworth Rd., Athlone.	4 yrs.	F.	-	-	-	Not Investigated.
Cornelia Alexander.	28 Cross Street, Cape Town.	4 yrs.	F.	-	-	-	Not Investigated.
Peter Frans.	46 Overton Rd., Zone B., Silvertown, Athlone.	4 yrs.	M.	-	-	-	Not Investigated.
Mogamat Isaacs.	9 Coburn Street, Cape Town.	4 yrs.	M.	Mrs. Isaacs.	Gr. Grand-mother.		Screened City Hosp. Has active T.B. Same Address.
Joseph Freeman	4 Hoskin Lane, Cape Town.	4 yrs.	M.	John Cruywagen.	Lodger.	28. 7.47.	
Hilda Smith.	14 Dryden Street, Salt River.	4 yrs.	F.	Freda Smith.	Mother.	24. 2.50.	
Martin Scott.	16 Dove Street, Salt River.	4 $\frac{1}{2}$ yrs.	M.	John Scott.	Father.	20.11.47.	
Kyben Salie	47 Scotsche Kloof, Cape Town.	5 yrs.	M.	-	-	-	Not Investigated.
Abdullah Achmat.	1 Nares Street, Observatory.	5 yrs.	M.	Gava Ismael.	Lodger.	15. 9.48.	Same Address.
Lional Swaine.	13 de Smith Str., Cape Town.	5 yrs.	M.	Victor Fernandez.	Lodger.	14. 1.50.	Same Address.
Ebrahim Snyman.	No. 16 Canterbury Str., Cape Town.	5 yrs.	M.	Dirk Bason.	Lodger.	1. 6.44.	Same Address.
Mary Johnson.	128 Glenboig Str., Perow.	5 yrs.	F.	-	-	-	Not Investigated.
Neville Collison	7 de Smith Str., Cape Town.	5 yrs.	M.	Sylvia Collison	Sister.	14. 9.50.	
Valantine Adams.	c/o Mr. Jordaan, Theunis Kraal, Tulbagh.	5 yrs.	M.	-	-	-	Not Investigated.

TABLE XVII (continued).

Coloured Patients.

Name.	Address.	Age.	Sex.	Pulmonary Tuberculosis Contacts.			
				Name.	Relation to Patient.	Notified as Pul. T.B. on	Remarks.
Harriet October.	12th Ave., Bellville South.	5 $\frac{1}{2}$ yrs.	F.	-	-	-	No Contact.
Moosa Maasdorp.	Klipfontein Rd., Athlone.	6 yrs.	F.	-	-	-	Not investigated.
Valerie Petersen.	Forrester Rd., Eerste River.	6 yrs.	F.	-	-	-	Not Investigated.
Grace Gordon.	Jasmine, Galway Rd. off Roscommon Rd. Healthfield	6 yrs.	F.	Dorothy Gordon.	Aunt.	28. 1.44.	Same Address.
Nora Phillips.	No. 13, 5th Ave., Bokmakierie.	6 yrs.	F.	Annie Phillips.	Mother.	-	Died 14.5.1949 of Pulmonary T.B.
Johanna Scholtz.	24th Ave., off Klipfontein Rd., Elsie's River.	6 yrs.	F.	-	-	-	Not Investigated.
Johannes Smit.	c/o Rev. Loodtz, Soetweide Str., Stellenbosch.	6 yrs.	M.	-	-	-	No Contact.
William Woolfe.	c/o Mr. Aandragh, de Bron Brackenfel.	6 yrs.	M.	-	-	-	Not Investigated.
Anita Christians.	22 da Gama Str., Brooklyn.	6 yrs.	F.	-	-	-	No Contact.
Ephraim Davids.	18 Frere Str., Cape Town.	6 yrs.	M.	Armina Kleinsmidt.	Lodger.	25. 3.49.	Same Address.
Asa Samsodien.	6 A Combrink Str., Cape Town.	7 yrs.	F.	-	-	-	No Contact.
Fatima Maxwell	58 Jordaan Str., Cape Town.	7 yrs.	F.	Alie Maxwell	Father.	5. 4.51.	-
Anna Bougat.	c/o Mr. le Roux, Burgundy Franchhoek.	7 yrs.	F.	-	-	-	Not Investigated.
Eileen Olivier.	52 Beattie Ave., Garden Village, Maitland.	7 yrs.	F.	-	-	-	No Contact.