Thesis

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An attempt to induce calcification and healing in experimental tuberculosis of guinea pigs

by

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[Signature]

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Introduction.  
Object.  
Acknowledgments.  

Historical introduction.  

Review of important literature.  

Present investigation  
(a) Method.  
(b) Results.  

Discussion. (Including previous investigations)  

Summary.  

Conclusions.  

Appendix.  

Bibliography.  

app. p. 73.
OBJECT.

The object of this study was to ascertain the effect of tuberculin, calcium, cholesterol and vitamins A and D, on the course of experimental tuberculosis in guinea pigs, and, by the use of these different therapeutic measures alone and in combination to discover the optimum method of inducing calcification and healing. It was considered desirable to employ these remedies in a manner which would be readily applicable to tuberculous disease in man. In order to give the therapeutic measures adopted as long a time as possible to influence the progress of the infection it was aimed to inoculate the guinea pigs with a sufficiently small dose of bacilli as would enable them to live several months.

The theoretical considerations upon which the experiment is based assume that healing is accompanied by the deposition of fibrous tissue, and that calcification is a form of healing, or at least that a calcified focus represents a less dangerous lesion for the further spread and dissemination of tuberculosis.
ACKNOWLEDGMENTS.

The present work was undertaken in the Laboratory at Southfield Sanatorium Colony, Edinburgh, during the tenure of a Research Scholarship from the Royal Victoria Tuberculosis Trust, and while acting as Clinical Assistant at the Sanatorium from July 1934 to July 1935.

I wish to express my grateful thanks to Professor Sir Robert Philip for his permission to submit this research done, at Southfield Sanatorium Laboratory, as a Thesis for the M.D. degree at Edinburgh University and for his frequent advice and interest during the course of this work.

I am much indebted to Colonel Harvey of the Royal College of Physicians Laboratory for the preparation and mounting of the sections, and to Doctor C.P. Stewart for advice given at the commencement of the experiment.
HISTORICAL INTRODUCTION.

The use of calcium in the treatment of tuberculosis is almost as old as our knowledge of tuberculosis itself. It is first recorded in Roman writings of the 6th century where, in Littrés Pliny we find that tallow from a horse mixed with lime is recommended in the treatment of scrofulus tumours. (1)

Medical practice in Rome at this time was largely founded on the teaching of Hippocrates and his contemporaries who had a relatively clear conception of tuberculosis from the clinical side. An abstract from the writings of Aristotle quoted by Galen proves that they were also aware of the association of calcification and tuberculosis, in animals at least. "The kidneys and the liver are frequently filled with calculi and tubercles and form pustules; the same things occur in the lungs, and, above all, in the spleen." Among the multitude of remedies advised at this period it is not to be wondered at that lime and ashes found a place. Whether greater specificity than that now claimed for the use of calcium in tuberculosis was held, and whether its use was prompted by the discovery of calcium in tuberculous lesions is however doubtful.

In the succeeding centuries little advance was made in the science of medicine, and in the 17th century/
century medical thought was still influenced greatly by the teaching of Hippocrates and Galen. Thomas Willis, Professor of Natural Philosophy and of Medicine at Oxford in his post-mortem descriptions mentioned "that whilst there was entire absence of ulceration of the lungs there were scattered through them in every part, tubercles or stones or sandy matter."

In the latter part of the 17th century Richard Morton wrote a book on 'phthisis' and amongst its causes we read "also chalkstones that are preternaturally bred in the lungs, or nails and other hard bodies slipping down into the lungs when persons laugh, are to be reckoned amongst the causes of consumption of the lungs." For the treatment of advanced consumption, chalk and coral amongst other remedies were recommended. He was aware of the association of calcification with chronic consumption of the lungs in scrofulous conditions. "Another characteristic of it, is its chronicity. The tubercles in the lungs in these cases are usually either crude or phlegmatic and not much disposed to inflammation or maturation. Sometimes they are seized with inflammation and the matter in them is cooked and hardened into a chalky or steatomatous substance, or into a honey like substance, and then the inflammation and exulceration of them is slow and almost insensible."

Morton accounted for the consumption by the chalk stones/
stones. He wrote "I have often observed chalky stones bred in the lungs which when they have been angular, and disturbed with the shaking of the lungs, are wont to tear the tender substance of those parts."

He also observed smooth chalkstones at autopsy 'without the least tubercle or ulcer occasioned by them'. Milk which has a large calcium content he warned against; "when it becomes evident that a stone has been formed in the lungs, a milk diet should be used cautiously lest it give rise to new chalk stones".

In a book published towards the end of the 18th century by Hufelund, physician to the king of Prussia, we find muriate of lime and lime water recommended in scrofula, and Thomas Beddoes in 1799 extolled the virtues of lime water in the treatment of tuberculosis.

Bayle in a book published in Paris in 1810 revealed that he was so familiar with calcification in tuberculosis that in his classification of phthisis into six different types he named one of them 'calcularous phthisis'. He stated "although very rare the calcaneous type of phthisis has nevertheless long since been described. The lung encloses concretions resembling, now small pieces of stone, again glomerules of chalk and sometimes particles of bone. Sometimes indeed it is completely stuffed with such concretions which are nearly always located in the bronchial glands or in small cysts, and sometimes between the bronchi/
bronchi or between the first divisions and the bronchial ramifications". Bayle described clinically the different types which accompanied his pathological classification and was aware of the association of chronicity and healing with his calculus type, but he did not appreciate the exact relationship between the pulmonary concretions and the tuberculous process.

With the advent of Laennec appear the first rays of light as to the significance of calcification and healing in tuberculosis. In writing of chronic and healed lesions he said "very often the development of these accidental cartilages is accompanied or followed by an abundant production of phosphate of lime in the neighbourhood; ..... quite often, too, the pulmonary tissue is infiltrated with the same substance, more or less dry, and mixed with black matter in the places previously occupied by tubercles".

Felix von Niemeyer writing in 1870 of caseous pulmonary lesions said "organic substances may disappear and calcaneous salts may become deposited until a cretaceous or mortar like concretion remains" and Villemin in his book 'Études sur la tuberculose' mentioned a form of healing in tuberculosis which ended in chalk formation.

An awakened interest in, and the dawn of a new conception of the relationship of calcium and tuberculosis, may be said to date from 1877 when Senator advanced/
advanced the theory that in tuberculosis there is an excessive excretion of calcium. Ferrier (2) in 1900 supported this 'demineralization' theory. His views obtained wide recognition, particularly in France, and evoked a keen and scientific interest in the study of calcium metabolism in tuberculosis. He pointed out that tuberculosis mortality was higher in France in those areas where the calcium content of the soil and water was least. Sergent (3) in 1910 gave a comprehensive survey of the literature on 'demineralization' in tuberculosis and expressed his agreement with Ferrier's views.

A further stimulus to the investigation of calcium in tuberculosis arose from observations of practical significance by Fisac in Spain in 1909 and Selkirk (4) that tuberculosis was almost unknown amongst workers in lime.

Within more recent years the advance in the knowledge of vitamins, their preparation in concentrated form, and the discovery of the relationship of vitamin D to calcium metabolism has stimulated a comprehensive research into calcium and the part it plays in tuberculosis, which has continued to the present day.

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A comprehensive survey of the literature within the scope of this work leads into a vast field covering many years of research and achievement. The discovery of vitamins A and D, and their functions, the metabolism of calcium and cholesterol, the action of tuberculin and the work done on these substances in relation to tuberculosis, presents a volume of literature to which it would be hard to do adequate justice. In consequence only the more important work will be referred to, and more particularly that which applies to the treatment of tuberculosis, and to the part played by calcium in that disease.
The rationale of Calcium Therapy.

Calcium therapy in tuberculosis is based on the finding of calcium in healed or latent lesions, often sterile, and commonly in disease of a chronic fibratic type.

Calcification is regarded as one of the end results of the constructive forces of healing and has suggested to many that calcium might be used therapeutically with this end in view. Others believe that the calcium deposition represents a general reaction to necrotic tissue. There is no conclusive evidence that calcification favours healing, rather than follows healing and Wells (5) writes that "calcification as it occurs in tuberculous areas does not differ from calcification in any other tissue. The calcium salts are laid down in tubercles according to the universal principle that necrotic tissues........ which cannot be absorbed will become impregnated with calcium salts." Sweany (6) stated that calcification may occur in any type of encapsulated tuberculous focus, and more especially in older lesions of this type. Whatever the divergence of views as to the significance of calcification there is general agreement that calcified lesions in the course of the disease represent less dangerous foci for further dissemination than do non calcified areas.

Hamburger and Tunnicliff (7) demonstrated that
calcium salts increased phagocytic activity, and calcium has been reputed indispensable in the processes of coagulation and fibrosis. (8)

'Demineralization' in Tuberculosis.

The theory that in tuberculosis there is a 'demineralization' has been widely held as justification for the employment of calcium in its treatment. This view has found wide support in France (3) but Wells (9), Kahn (10) and Russel (11), in discussing it, expressed doubt as to its occurrence. It is generally agreed that in certain types of tuberculosis there may be a calcium loss. For instance where there are intestinal lesions and diarrhoea there may be a negative calcium balance (12) and Laufer stated (13) that where exudative lesions predominated there was an increased elimination of calcium. This important question of 'demineralization' in tuberculosis will not be referred to further at the present stage but will be amplified in the 'discussion'.

Calcium Metabolism.

The absorption and utilization of calcium, its distribution and chemical formation in the blood serum, and the relationship of the parathyroids, however important in any discussion of calcium metabolism, will not be elaborated here. Briefly it may be stated that the normal absorption of calcium depends not only on the quantity ingested but also
on the gastric hydrochloric acid content, the intestinal reaction, and the amount of phosphate present in the diet. There is a definite optimum proportion for calcium and phosphorous and this is said to be regulated by vitamin D in a manner which is at present uncertain. Vitamin D is also said to influence the reaction of the intestinal contents and so has an important bearing on the absorption of calcium. Bergheim (14) stated that in his opinion vitamin D acted not so much by increasing the absorption of calcium but its retention. Stewart and Percivall (15) concluded that more probably it acted by controlling the pH of the intestine, either by stimulating the secretion of hydrochloric acid in the stomach or inhibiting the flow of alkali in the intestine. Crimm (16) stated that viosterol (vit.D) increased the absorption of calcium as indicated by large the elevation of serum and urinary calcium and doses increased the absorption and retention of calcium in the tissue fluids to effect a state of hypercalcaemia. Taylor and Weld (17) believed that vitamin D delayed the excretion of calcium into the gut and hypercalcaemia produced by it is due to the withdrawal of calcium from the skeleton. Normally the blood is practically saturated with calcium to the limit of its carrying power, and therefore does not take up more calcium from the intestines. Even if excess amounts of calcium are introduced into the blood by intravenous injection the normal concentrations are re-established in a very short time.
Clinical use of Calcium in Tuberculosis.

Numerous investigators have tried the effect of the oral administration of calcium salts on blood calcium values and in treating tuberculosis. Brockbank (18) stated that calcium by mouth failed to alter the serum calcium materially. He found that serum calcium values were on the average slightly lower in cases of advanced pulmonary tuberculosis than in normal subjects, and slightly higher than normal in cases of pulmonary tuberculosis, healed or healing. Stewart and Haldane (19) obtained a temporary but definite rise, 10-20 per cent in serum calcium values by oral administration. Denis and Minot (20) stated that the result of a study of the effect of administering calcium salts by mouth to men, rabbits and cats, indicated that it was impossible in most cases to increase the concentration of calcium in the plasma by ingestion of calcium salts, and their results were corroborated by Halverson and Bergeim (21). Kahn and Ræe (22) found in men that 5 G. and 2 G. doses of calcium lactate produced increases in serum calcium values of 80 per cent and 41 per cent respectively, while Bauer and Ropes (23) reported only 8 per cent increase after 5 G. calcium lactate and 14 per cent increase after 10 G. A maximum rise occurred in 1-5 hours and in 7 ex 8, the serum calcium had not returned to normal levels in 12 hours. Hein (24) gave clinical reports of the favourable use of calcium by mouth in tuberculosis.
Calcium in experimental Tuberculosis.

Experimentally calcium has been tried for its effect on tuberculosis and on blood calcium values. Hoyle (25) found in rabbits that single and repeated doses of calcium lactate given orally in doses of 1 G. per kilo. body weight increased the serum calcium 15-22 per cent and that calcium carbonate employed in the same manner gave increases of 20-25 per cent. Laitinen (26) reported two series of experiments, each in 20-30 infected guinea pigs, in which animals which were fed on calcium phosphate lived twice as long as controls. Mawer and Wells (27) investigated the effect of calcium administration on tuberculous guinea pigs and concluded that this procedure did not increase calcium deposition, did not reduce the amount of spread of tuberculosis, nor lead to fibroblastic tissue reaction. However they found that the extent of the lesions in the lymph nodes was much greater in 7 ex 9 in the controls, and in the spleens in every case the extent of the tuberculosis was much greater in the controls than in the calcium fed guinea pigs. The average length of life of the calcium fed guinea pigs was 92.5 days while that of the controls was 74.4 days but they attach no significance to this. The weight curves showed no appreciable difference.
The effect of vitamin D in treating experimental tuberculosis and on calcification.

Harris and Moore (28) and Kreitmor and Moll (29) have shown that vitamin D in excess causes a rise in blood calcium and deposition of calcium in certain organs, especially aorta and kidney. Spies (30)(31) showed that the caseous lesions of experimental tuberculosis in rabbits calcify more extensively when they have received repeated large doses of irradiated ergosterol, and Simmonet and Tanret (32) showed by chemical analysis that lung tissue of both healthy and of tuberculous rabbits contained much more calcium after large amounts of irradiated ergosterol. Loewy and Gruninger (33) used vigantol and calcium in the treatment of tuberculosis in guinea pigs and concluded it produced no favourable results, though in their experiments large and toxic doses of vigantol were used. Spies and Berryhill (34) treated experimental intra-abdominal tuberculosis with large doses of irradiated ergosterol and produced calcification of intra-abdominal tubercles. No tuberculous lesions were found in the kidneys of the infected animals but in spite of that calcium was deposited there to a much more marked degree than in the kidneys of normal animals receiving still larger doses of vitamin D. The authors suggest that there may be some underlying factor which is related to the tuberculous process and affecting calcium metabolism.
Smith (35) used vitamin D as cod liver oil in treating experimental tuberculosis in guinea pigs but found it of no definite benefit as regards the weight curve, the length of life, or the extent of disease, and the addition of calcium caused no increased deposition in the lesions. Grant (36, 37) in studying the effect of vitamin deficient diets on tuberculosis in white rats found they were made susceptible to tuberculosis by decreasing the calcium and vitamin D in the diet. Jampolis (38) used large doses of irradiated ergosterol in experimental tuberculosis in guinea pigs and produced hypercalcaemia and calcification of necrotic tubercles. Zeyland (39) used vitamin D corresponding to human dosage in treating tuberculosis in guinea pigs as it seemed of no practical value should increased calcification occur after large and toxic dosage. He found a lack of notable difference in duration of life nor did post mortem examination reveal any macroscopic or microscopic difference in the extent of tuberculous lesions or in the quantity of histologically detectable calcium. Loewen and Oatway (40) tried the effect of irradiated milk on experimental inhalation tuberculosis in guinea pigs. They concluded that vitamin A and D, alone or together, did not exert any noticeable influence on the calcium content of the lungs of tuberculous or non-infected guinea pigs, and that calcium in the diet was not a factor in limiting the amount of tuberculosis; it did not increase the calcification of the lesions.
nor lead to fibroblastic tissue reaction. Compared with their control groups, they stated "that no remarkable prophylactic or therapeutic effects were found as judged by the extent or type of the resultant lesions or by the mortality statistics.

Clinical results of the use of Vitamin Treatment in Tuberculosis.

Clinically, vitamin D has received a thorough trial in the treatment of tuberculosis and it may be worth referring to a few of the results. Becker (41) reported improvement and X-Ray evidence of healing of 'open tuberculosis' under protracted treatment with vigantol and calcium but no evidence of calcification was found. Similar clinical observations on the effect of viosterol on pulmonary tuberculosis were made by Kaminsky and Davidson (42). They concluded that while the oral administration of small doses of irradiated ergosterol caused a rise of the serum calcium in patients with pulmonary tuberculosis, it did not seem to influence to any extent the degree of calcification of lung lesions so far as detectable by X-Ray. Poncher and Gasul (43) in observations on the use of vitamins in tuberculous children questioned the use of moderately large doses of vigantol and could draw no conclusion as to it having any effect on the course of the disease. They warn against the secondary effects of such therapy though this has no bearing on the provision of normal
adequate vitamin D requirements in the diet.

Grimm (44) used vitamin D in the treatment of pulmonary tuberculosis and stated "we feel that activated ergosterol may favour fibrosis and hasten absorption of tuberculous infiltration in pulmonic tissue when opportunely administered." Large amounts of vitamin A and D had no recognisable effect in cases of bone tuberculosis treated by Paterson (45).

Tuberculin in the treatment of experimental tuberculosis.

The literature on the use of tuberculin as a therapeutic agent in the treatment of experimental tuberculosis is very extensive and only a few results will be referred to here. Savitch et al (46) stated that guinea pigs treated with ascending doses of tuberculin (O.T.) and irradiated ergosterol presented a pathological picture which differed from that found in animals treated by either alone. The guinea pigs receiving combined treatment demonstrated greater longevity, less extensive macroscopic tuberculosis, and a microscopic picture in which the tuberculous lesions appeared to be more circumscribed, less caseous, and more fibrotic than that in the animals receiving either irradiated ergosterol or tuberculin alone. The same observers (47) treated tuberculous rabbits with tuberculin, vitamin D, parathormone and by calcium gluconate given intra-
intramuscularly. They obtained benefit in certain groups and stated that any agent or group of agents exercised its maximum beneficial effect only in the presence of tuberculin. The viosterol, calcium, and tuberculin group were striking in showing marked calcification and fibrosis and animals killed were in better condition than in any other treated group. They found no benefit in the group treated with tuberculin only. Philip (48) who has emphasised the especial therapeutic qualities of Beraneck's Tuberculin (T. Bk) found distinct and striking improvement as regards longevity in guinea pigs inoculated with tubercle bacilli (Human) when treated preventively and therapeutically with this tuberculin. Differences up to 100 days in longevity were noted as compared with untreated controls. Sewall (49) found the preliminary administration of tuberculin given intravenously to infected guinea pigs raised their resistance and Corper (50) stated that tuberculin (Kochs 0.T.) had no appreciable effect upon the course of tuberculous infection (Human) in the guinea pig.

**Cholesterol in the treatment of Tuberculosis.**

It was decided to employ cholesterol in combination with calcium and vitamin in the present investigation. The reasons which suggested its possible therapeutic efficacy were the high cholesterol content of brain tissue which is used with
some benefit in the treatment of pulmonary tuberculosis (51) and because of the high cholesterol content of necrotic tissue which precedes calcification, both in tuberculosis and in other disease. (52). Sweany (53) stated that in general the higher the cholesterol content of the blood the greater the resistance, and Eisler and Laub (54) found evidence of a decrease in blood cholesterol with progressive disease. Hinz (55) also found that even in the early stages of acute tuberculosis with poor prognosis there is a decrease in blood cholesterol. Jaffe and Levinson (56) treated experimental tuberculosis in the rabbit with cholesterol and stated that oversaturation of the body of the rabbit with cholesterol does not protect the animal against tuberculosis. Henning (57) concluded from his cholesterol studies in tuberculosis that it may be important in the defensive reactions of the body and Shope (58) testing its possible therapeutic effects thought that some prolongation of life occurred in acute infections. Beumer (59) also noticed a protective action by cholesterol when administered to guinea pigs injected with diptheria toxin and he thought deposits of cholesterol in reticulo endothelium may increase resistance. Wade (60) in studies on calcium and cholesterol in thyroparathyroidectomised dogs concluded that there was a definite relationship between these two substances.
The preceding literature reveals considerable divergence of views as to the therapeutic activity and effects of calcium and vitamin D.

Oral administration of calcium was found to raise the level of serum calcium by Stewart and Haldane, Kahn and Roe, Bauer and Ropes, Salvesan (69) Jansen (70) and Hoyle, while Brockbank, Denis and Minot, Halverson and Bergeim, Clark (71), Kramer and Howell (72), deny such effect.

The important influence of vitamin D on the absorption of calcium is an acknowledged fact. There is general agreement that in excess it caused a rise in serum calcium values and the majority found that in excess it increased calcium deposition. Smith, Zeyland, and Loewen and Oatway stated that in therapeutic doses it lacked any such influence.

Becker, Kaminsky and Davidson, and Grimm found clinical improvement under vitamin treatment, and Pattison and Poncher and Gasul said no benefit was derived from this therapy.

The majority found calcium and vitamin of little service in the treatment of experimental tuberculosis. Mower and Wells, Loewy, Smith, Zeyland, Loewen and Oatway, and Savitch employed calcium and vitamin D, alone or combined, in the treatment of experimental tuberculosis and they stated no therapeutic benefits were derived from their use, while Laitinen Jampolis and Levaditi and Po (73) claimed favourable effects.
PRESENT INVESTIGATION.

The nature of an experimental study on the lines to be described implies a careful consideration and control of all factors likely to have an influence upon the health of the animals. Environmental conditions must be suitable and uniform, incidental infections must be guarded against, controls must be provided, and the dosage of bacilli inoculated into the different animals must be as nearly uniform as possible.

The guinea pigs used in these experiments were all raised in the breeding establishment at Southfield Sanatorium Laboratory. They were housed some distance away from the building where the actual experiment was carried on and every precaution was taken to prevent spontaneous tuberculosis in these animals. In addition all were observed for two weeks before inoculation and each animal before being used in the experiment was given an intracutaneous tuberculin test of 0.1 c.c. of a 1:1000 dilution of tuberculin (O.T.) prepared in the Laboratory of the Royal College of Physicians, Edinburgh, and none were found to give a positive reaction after 48 hours.

The stock was healthy and there was no evidence of incidental infection amongst the animals. Thirty-six guinea pigs were used, divided into six equal groups/
groups. Animals of the same sex were put into cages, 6 or 3 in a cage according to its size, and were kept in a building where the temperature during the course of the experiment was kept at approximately 60°F. An electric radiator was used for heating the room during the winter months. Abundance of fresh air was provided and no direct ultra-violet rays were allowed access to the cages. The animals, apart from the special treatment were fed on the stock diet normally provided. This consisted of a bran mash daily, with turnip and hay in winter and fresh greens in the summer. Twice a week oats were given. Each cage was fitted with a shallow zinc trough from which the animals fed.

The various drugs used, with the exception of tuberculin, were incorporated in the diet as it was felt that this method was preferable as it entailed no unnecessary handling of the animals and was more readily applicable to the treatment of tuberculosis in humans. They were thoroughly mixed in the individual troughs with the bran meal given in the morning and, of the bran mixture, sufficient only was given to ensure that the meal would all be finished.

The animals were divided into 6 groups and following inoculation with tubercle bacilli were treated as follows:

Group/
Group A. Calcium and tuberculin.
Group B. Calcium, cholesterol, vitamin and tuberculin.
Group C. Tuberculin.
Group D. Calcium, vitamin, and tuberculin.
Group E. Calcium, cholesterol and tuberculin.
Group F. Controls - infected and no treatment given.

Inoculation of guinea pigs with tubercle bacilli.

The suspension was prepared from a six weeks old culture of a known virulent human. (Inman) strain received at Southfield Sanatorium Laboratory and grown on glycerin egg medium. (29/10/34). After being ground in an agate mortar the suspension was filtered through sterile No.1. Whatman paper and diluted with saline until it formed an opalescent solution just visible to the eye. This was further diluted 20 times and thoroughly mixed.

A haemocytometer count indicated that each c.c. of diluted suspension contained approximately 1,000,000 tubercle bacilli.

Bacilli were seen lying singly and in small clumps in films made from the diluted suspension.

Of the resulting diluted suspension .1 cc. was inoculated subcutaneously into the left groin of the guinea pigs.

The animals being 'unsensitised' the disease following this injection was therefore of the primary infection type.
Administration of Vitamin.

Vitamins A and D were given combined in the form of Adexolin obtained from the Glaxo laboratories. It was decided to utilize vitamin A in the experiment to ascertain if its anti-infective properties (61) would have any recognisable influence on the progress of the tuberculous infection and in Adexolin we have a convenient combination of the two vitamins.

The amount necessary was to some extent problematical but it was felt desirable to ensure that the dose given was non toxic. Guinea pigs do not normally consume any vitamin A at all, being dependent upon their own ability to metabolise this from the carotene present in the green leaves of their normal diet. The daily prophylactic requirement of young rats weighing from 50 - 100 grammes can safely be taken as 5 international units of vitamin A and 2 international units of vitamin D. (62). Calculating on a weight-for-weight basis, a guinea pig should require five times this amount, that is 25 units of A and 10 units of D. Adexolin liquid contains in every c.c. 12000 units of A and 2000 units of D. To ensure an adequate dosage and to allow for any unevenness in the mixing and consumption one drop of Adexolin per animal was given daily, that is 400 units of A and 70 units of D. Throughout the experiment no toxic effects from this dosage were manifested.
Administration of Calcium.

Calcium was given in the form of calcium lactate. To ensure the maximum absorption of calcium, in addition to adequate vitamin D, the calcium phosphorous ratio must be as nearly correct as possible. Sodium phosphate was mixed with calcium lactate in the powders given in the diet. The powders contained 7 grains of calcium lactate and 3 grains of sodium phosphate per guinea pig. (63)

Administration of Tuberculin.

The tuberculin, (K 24) used in treatment was Koch's Old Tuberculin (Human) obtained at the Laboratory of the Royal College of Physicians, Edinburgh, and standardised at Southfield Sanatorium. The tuberculin was given in ascending doses once a week, a fresh dilution in saline being made on each occasion. The method of dilution was as follows:

.1 c.c. of tuberculin was added to .9 c.c. saline giving a solution of 1 c.c. of 1/10 tuberculin. This was added to 9 c.c. of saline giving a 1/100 solution and after thorough mixing, 1 c.c. of this solution was added to another 9 c.c. saline and so on until a dilution of 1 in a million tuberculin was reached. Of this dilution \( \frac{1}{8} \) c.c. was given for the initial injection. The injections were given subcutaneously in the flanks. A severe reaction at the site of the injection/
injection or signs of increased malaise amongst the animals after injection was taken as an indication that the dose given was excessive and the following injection was not increased or was even reduced.

It was found in the course of the experiment that 1 c.c. of 1/100,000 tuberculin was the maximum dosage tolerated and no dose greater than this was administered.

Cholesterol was given in powders mixed with the food and those animals receiving it were given a daily dose of five grains.

Method of recording observations.

The guinea pigs were weighed weekly and at the same time the injection of tuberculin was given to avoid needless handling. They were weighed on the same day each week and before the morning feed was given.

The average weights of the animals in each group were recorded and are shown in Table 1. The details of the weight progress in each group are given in pages 64--69, of the appendix.

The group average weights every three weeks were plotted in a curve and are shown diagramatically in Figure 1. The average loss of weight and the percentage loss of weight were similarly recorded - Figures 2 and 3.
The animals were weighed after death and autopsied as soon as possible. The post-mortem weights are shown in Table I.

The guinea pigs were opened and the presence or not of peritoneal, pleural or pericardial effusions noted. Observations were made on the distribution and type of the disease, on its extent, and a search made for possible signs of calcification.

In every case a smear was made from an infected organ, usually one of the lymph nodes, and stained with Ziel Nielson to make certain the pathological changes seen were caused by tuberculous infection. In every case acid post bacilli, presumably tubercle bacilli, were found.

The extent and type of disease in the liver, spleen and lung was noted. The amount of macroscopic tuberculosis observed was measured in terms of a predetermined standard, representing different degrees of tuberculous involvement in the different organs and conveniently represented by the symbols, +, ++, +++; a maximum of three units being allowed for the severest disease. The results are shown in Tables X-XI.

The area of the spleen in square centimetres was measured. The results are given on pages 53, & 54.

Especial attention was devoted to the lymphatic system. The lymph node involvement was noted in detail and the lymph nodes measured in centimetres.
The results are given in tabular form, Tables IV-IX, units being allocated to the nodes according to their size in centimetres.

The condition of the ulcer at the site of inoculation, in the course of the experiment and at post-mortem examination is recorded in Tables XV-XVI.

The survival period of the different animals in each group is shown in Table II and the group longevity is represented diagramatically - Figure 4.

At post-mortem a portion of spleen, liver and lung and a lymph node were removed and immediately transferred to Zenkers fixing fluid. The Zenkers fluid was made up without acetic acid as this is known to dissolve calcium.

Sections were prepared from these organs and stained by haematoxylin and eosin and by van Giesons connective tissue stain. The type of disease, the presence or not of calcification, and the degree of fibrosis, were observed microscopically.

The presence of calcification is recorded in Table XIV and the degree of fibrosis in Table XII.

The post-mortem findings in each animal and the microscopic description of the sections are given in the appendix.

Summarised, as a means of therapeutic evaluation, the method of recording the course and pathology of the tuberculous infection in the different groups is as follows:-
S factor. (1) Weight progress.
(2) Survival period.

L factor. (3) Macroscopic lymphatic system disease.
(4) Macroscopic liver, spleen and lung disease.
(5) Splenic index.
(6) Microscopic fibrosis.
(7) Presence of calcification.
(8) Primary inoculation.
(9) Post-mortem and microscopic description.
WEIGHT PROGRESS.

Date of inoculation with tubercle bacilli 14/12/34.

TABLE I.

Average weight of each group in grammes.

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<td>523</td>
<td>582</td>
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</table>

AVERAGE LOSS OF WEIGHT OF EACH GROUP IN GRAMMES.

<table>
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<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<tr>
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<td>165</td>
<td>86</td>
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<td>111</td>
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A. Calcium and Tuberculin
B. Calcium, Cholesterol, Vitamin and Tuberculin.
C. Tuberculin.
D. Calcium, Vitamin and Tuberculin.
E. Calcium, Cholesterol and Tuberculin.
F. Inoculated Controls.
Weight progress of each group in grammes.

Time in weeks.

A Calcium and tuberculin.
B Calcium, cholesterol, vitamin & tuberculin.
C Tuberculin
D Calcium, vitamin, & tuberculin.
E Calcium, cholesterol & tuberculin.
F Innoculated.
Average loss of weight in each group in grammes.

A Calcium & tuberculin.
B Calcium, cholesterol, vitamin, & tuberculin.
C Tuberculin.
D Calcium, vitamin, & tuberculin.
E Calcium, cholesterol, & tuberculin.
F Innoculated controls.
Figure 3.

Percentage loss of weight in each group.

- A Calcium & tuberculin.
- B Calcium, cholesterol, vitamin & tuberculin.
- C Tuberculin.
- D Calcium, vitamin, & tuberculin.
- E Calcium, cholesterol, & tuberculin.
- F Innoculated controls.
The observations of De Witt (64) on the weight curves in tuberculous guinea pigs have indicated that they may be of some value in estimating the influence of treatment on the disease.

Table I and Figure 1 record the average weights of the different groups at 3 week intervals. Figure 1 records the weight progress for 21 weeks only, as after this period the incidence of deaths would affect the accuracy of such an estimate as the group average weight. The guinea pigs were weighed after they were removed from the breeding enclosure to the cages and before they were inoculated with the tubercle bacilli.

The weight progress of the different groups are approximately parallel, though several differ in minor detail. The majority show a rise in weight for the first 6 weeks though there is initially a slight loss of weight in the control group. The initial rise in weight is probably due to the better feeding and attention received when being fed in the cages, to the more uniform temperature of the animal house, and to the lack of exercise. Mayer & Wells (27) similarly noted a rise of weight in tuberculous guinea pigs in the few weeks following inoculation.

At from 6 to 9 weeks the progress of the infection begins to have affect and there is a gradual loss of weight. This period also coincides with the progress of/
of open ulceration at the site of inoculation.

The control group after the initial fall shows a steady rise in weight until the 12th week, when the weight remained practically stationary until the 18th week. In group E - calcium, cholesterol and tuberculin - it will be seen there is a slight rise in weight from the 6th to the 12th week.

The average loss of weight in each group, as one would expect, depends largely on the initial weight and it will be seen in Table I that at time of death the weights are approximately the same. The greatest loss of weight occurred in groups F and A which had the greatest average weight at the commencement of the experiment.

The greatest gains in weight after inoculation are found in group D, - calcium, vitamin and tuberculin, and group C - tuberculin - but as these gains occurred comparatively early in the course of the infection it can hardly be assumed that the treatment given in these groups was at any rate wholly responsible for this.

The best sustained weight curves are found in group E - calcium, cholesterol and tuberculin, and in group F, - the controls.

From these results one could not state with assurance that the treatment given to the different groups had any beneficial influence on the course of the/
the tuberculous infection. The control group receiving no treatment at all shows the most favourable weight progress curve but the fact that the dose of bacilli relative to their weight was smallest of all may account largely for this finding. Krause (65), in experiments on the resistance of young and old guinea pigs found that when injected subcutaneously with large quantities of virulent tubercle bacilli in equal doses, a more extensive and progressive involvement resulted in young than in old animals.

The young received for body weight double the dose and he attributed this result as probably due to overdosage.
LONGEVITY IN DAYS.

**TABLE II.**

<table>
<thead>
<tr>
<th></th>
<th>A</th>
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</table>

A. Calcium and tuberculin.
B. Calcium, vitamin, cholesterol, and tuberculin.
C. Tuberculin.
D. Calcium, vitamin and tuberculin.
E. Calcium, cholesterol and tuberculin.
F. Controls.
Average duration in days of each group.

- Including early incidental deaths.

A  Calcium & tuberculin.
B  Calcium, cholesterol, vitamin & tuberculin.
C  Tuberculin.
D  Calcium, vitamin, & tuberculin.
E  Calcium, cholesterol, & tuberculin.
F  Inoculated controls.
Comparison of initial weight with survival period.

**TABLE III.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Average survival period in days</th>
<th>Average weight in grammes</th>
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<td>E</td>
<td>184</td>
<td>633</td>
</tr>
<tr>
<td>F</td>
<td>192</td>
<td>762</td>
</tr>
</tbody>
</table>
Comparison of initial weight with longevity.

Red = Weight.

Green = Survival period.

A Calcium & tuberculin.
B Calcium, cholesterol, vitamin, & tuberculin.
C Tuberculin.
D Calcium, vitamin, & tuberculin.
E Calcium, cholesterol, & tuberculin.
F Controls.
Figure 4 shows diagrammatically the average duration in days of the different groups, and the number of days which each animal survived after inoculation is given in Table II.

Survival period is perhaps an uncertain criterion for judging the effect of treatment on virulent tuberculosis in guinea pigs. The disease becomes generalised in all, as quoted from Krause in the previous paragraph weight and age of animals are important factors influencing the progress of the disease. Unknown factors governing individual resistance and susceptibility will also affect the longevity of infected animals and be particularly evident when dealing with small groups as in the present experiment. Lewis (66) stated that in guinea pigs he has yet to conduct an experiment in which the last animal to die did not live at least twice as long as the first to die.

It was hoped, that the longer the animals lived, any effect of treatment would become more obvious. The aim in the present experiment as stated earlier was to give such a dose of bacilli as would enable the guinea pigs to survive approximately 6 months. This was largely achieved and as will be seen from Figure 4 in only one group was the average survival period below 6 months. There is considerable individual variation/
variation in longevity as would be expected but in only two cases of early death could this be attributed to incidental infection. Apart from these two cases occurring relatively early in the course of the experiment, death in all other cases appeared to have been due to the tuberculous infection as found post mortem.

From the Figure 4 it is seen that no very striking difference in longevity is noted between the different groups. With such small groups any marked variation in the resistance of even one animal will markedly alter the average longevity for that group.

The longest survival period is found in group D - calcium, vitamin, and tuberculin, with next in order group F - the controls.

In two groups, group E - calcium, cholesterol and tuberculin, and group C - tuberculin, all the animals survived to 170 days and longer.

An attempt was made to ascertain if any relationship existed between the survival period and the weight at commencement of the experiment. The comparison is shown in Figure 5 and Table III. It will be seen that the two groups with the longest average survival period - group D and group F, had two of the highest initial weights. Group A on the other hand with the second greatest average weight had the shortest/
shortest duration of any of the groups. As a result of the comparison made in these small groups, no conclusive relationship exists between weight and longevity.
**LYMPH NODE INFECTION.**

### TABLE IV.

<table>
<thead>
<tr>
<th>L.Inguinal</th>
<th>R.Inguinal</th>
<th>L.Lumbar</th>
<th>V.Lumbar</th>
<th>Mesenteric</th>
<th>Portal</th>
<th>Cervical</th>
<th>Axillary</th>
<th>Mediastinal</th>
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</table>

*+ = 1 cm. and less*

*++ = 2 cm. and less*

*+++ = 2 cm.*

**Group A.** Calcium and Tuberculin.

**Group B.** Calcium, cholesterol, vitamin and tuberculin.
## LYMPH NODE INFECTION

### TABLE VI

<table>
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### TABLE VII

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</tbody>
</table>

- + = 1 cm. and less
- ++ = 2 cm. and less
- +++ = 2 cm.

**Group C.** Tuberculin only.

**Group D.** Calcium, vitamin and tuberculin.
### LYMPH NODE INFECTION.

#### TABLE VIII.

<table>
<thead>
<tr>
<th>Group</th>
<th>L. Inguinal</th>
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#### TABLE IX.

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<th>V. Lumbar</th>
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</tr>
</tbody>
</table>

* + = 1 cm. and less
  
* ++ = 2 cm. and less
  
* +++ = 2 cm.

Group E. Calcium, cholesterol and tuberculin.

Group F. Inoculated controls.
It was hoped that any salutary effect of the various treatments used in the different groups would be revealed in the lymphopoietic system. The preceding tables represent in a convenient form for comparison the enlargement of the lymphatic nodes in the different guinea pigs. Particular attention was paid to the lymphopoietic system and the measurement in centimetres of the different nodes were noted at the post-mortem examination in each animal. At the same time the consistency, and presence or not of caseation were noted. It was assumed that the enlargement of the nodes in each case was due to the tuberculous infection, and it may be noted that in every case the lymph node removed for microscopic section in each animal, revealed tuberculosis.

Reference to the tables shows that there were but the smallest differences in the degree of lymph node enlargement between the various groups, and, as a method of assessment of treatment, it revealed no distinctive benefit as between that given to one group and another, and no group showed any noticeable benefit as compared with the control group. Such slight differences as were present revealed the least node enlargement in the group treated by tuberculin alone and in group E - calcium, cholesterol and tuberculin, while greatest node enlargement was found in group A - calcium and tuberculin. It is seen from the tables that/
that the greatest and most consistent degree of enlargement was found in the nodes draining the site of primary inoculation in the left thigh. The mediastinal nodes are next in size and uniformity of enlargement, with the portal glands slightly less so.

In 14 animals out of 34 the right inguinal nodes are not enlarged and in 16 there is no enlargement of mesenteric nodes. The axillary nodes are enlarged in 12 of the animals, the left axillary in 8, the right axillary in 3 and in one both right and left are enlarged.

Analysis of the tables, demonstrates a fair amount of discrepancy between the degree of lymph node enlargement of individual animals within each group. The earlier animals to die in each group, with the exception of group C. showed a definitely lesser degree of node enlargement than did the later. This is what might be expected, as the less the resistance of the lymphatic barrier, the less is the lymphatic reaction and the sooner a generalised spread with early death of the animal.

A comparison was made between those animals which exhibited calcification and the degree of lymph node enlargement and no relationship was revealed.
MACROSCOPIC INVOLVEMENT OF LIVER, SPLEEN AND LUNG.

<table>
<thead>
<tr>
<th>TABLE X.</th>
<th>TABLE XI.</th>
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<td>Liver</td>
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<tr>
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</tr>
</tbody>
</table>

+ = slight; few caseous nodules not greater than .5 cm.
++ = moderate; 6 or more caseous nodules .5 cm. in size.
++++ = severe; large and many caseo-necrotic patches, or miliary involvement.
Analysis of Tables X and XI, is of little help in differentiating the effects of treatment in the various groups. The representation by symbols of macroscopic disease in a series of animals which have died from an advanced and universally severe tuberculous infection can be but an arbitrary procedure. The measurement of the amount of tuberculosis is only relatively uniform as certain factors may operate that increase the amount of tuberculosis while prolonging the animals life, while others that diminish the amount of tuberculous pathology may at the same time lessen the period of survival. Certain organs showing large caseous patches are not necessarily more severely affected or more dangerous to the health of the guinea pig, than others which are riddled with miliary tubercles not obvious to the naked eye and perhaps giving rise to little enlargement.

The tables reveal little difference between the various groups. Group C - tuberculin, - on the whole would seem to reveal the most severe involvement and group A - calcium and tuberculin, the least, with the other groups approximately the same.
SIZE OF SPLEEN.

GROUP A. CALCIUM AND TUBERCULIN.

(1) 3.5 x 1.5 = 5.25 sq. cms.
(2) 6.7 x 3 = 20.1 " "
(3) 7.3 x 3.8 = 28.34 " "
(4) 4.2 x 2.2 = 9.24 " "
(5) 5.1 x 2.3 = 11.73 " "
(6) 4.5 x 2.8 = 12.6 " "

Average size of Spleen 14.21 sq. cms.

GROUP B. CALCIUM, CHOLESTEROL, VITAMIN AND TUBERCULIN.

(1) 5.5 x 2.3 = 12.65 sq. cms.
(2) 5.5 x 3 = 16.5 " "
(3) 6 x 3.6 = 21.6 " "
(4) 5 x 2.7 = 13.5 " "
(5) 5.5 x 3.7 = 20.0 " "
(6) 5.8 x 3.2 = 18.56 " "

Average size of Spleen 17.1 sq. cms.

GROUP C. TUBERCULIN.

(1) 4.5 x 2.6 = 11.7 sq. cms.
(2) 5.5 x 2.3 = 12.65 " "
(3) 4.8 x 2.2 = 10.56 " "
(4) 6 x 3 = 18 " "
(5) 7 x 3.5 = 24.5 " "

Average size of spleen 15.5 sq. cms.

GROUP D. CALCIUM, VITAMIN AND TUBERCULIN.

(1) 5 x 2.8 = 14 sq. cms.
(2) 5 x 2.5 = 12.5 " "
(3) 4 x 7 = 28 " "
(4) 5 x 2.5 = 12.5 " "
(5) 4 x 2.5 = 10 " "
(6) 4 x 2.2 = 8.8 " "

Average size of Spleen 13.4 sq. cms.
GROUP E.  CALCIUM, CHOLESTEROL AND TUBERCULIN.

(1) 5 x 2.6 = 13 sq. cms.
(2) 6.5 x 2.8 = 18.2 " "
(3) 3.8 x 2.3 = 8.74 " "
(4) 5.3 x 2.5 = 13.25 " "
(5) 6 x 3 = 18 " "
(6) 4.5 x 2 = 9 " "

Average size of Spleen 13.3 sq. cms.

GROUP F.  INOCULATED CONTROLS.

(1) 4 x 2.5 = 10 sq. cms.
(2) 5 x 2.5 = 12.5 " "
(3) 6 x 3.5 = 21 " "
(4) 5.2 x 2.5 = 13 " "
(5) 5.5 x 3 = 16.5 " "
(6) 4 x 1.9 = 7.6 " "

Average size of Spleen 13.4 sq. cms.
In guinea pigs the spleen develops more tuberculosis following subcutaneous inoculation than other organs, with next in order the liver, and the lung is last to develop tuberculosis (67). At different periods after subcutaneous inoculation the various organs show a different amount of tuberculosis. When the animal is allowed to die of the infection little variation in the amount of tuberculosis in the organ is seen, and, as in the present experiment, massive involvement of liver, spleen and lung is frequent.

Renche and Moore (68) emphasize that the spleen can be used in the course of tuberculous infection and when it shows changes, as an approximate index of the total tuberculous involvement of the guinea pig.

The preceding tables of splenic size as measured approximately by the length in centimetres multiplied by the breadth reveal but slight differences between the various groups. Group E - calcium, cholesterol and tuberculin, has the smallest average size of spleen, group F - the controls is but a shade larger, and group B has a definitely larger size of spleen than any of the other groups. These results indicate that as judged by splenic size no treatment manifested any advantage over the others or over the controls.

While there is little difference between the average size of spleen for each group, there are marked variations between the size of spleen in individual/
individual animals within each group. In each group one or more of the guinea pigs have a spleen markedly larger than that in the remainder of the group. In view of the above statement that the spleen can be used as an approximate index of tuberculous involvement a comparison was made between the splenic size and the macroscopic findings in liver, spleen and lung - Tables X - XI.

The result of the comparison contradicts this statement on the whole and is no doubt due to the guinea pigs being allowed to die of tuberculous disease which was almost universally gross in extent. It is possible that at an earlier stage of the disease the spleen would serve as an index of tuberculous involvement as stated by Renoch and Moore.
ESTIMATION OF FIBROSIS. VAN GIESONS STAIN.

<table>
<thead>
<tr>
<th>GROUP A.</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
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</thead>
<tbody>
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<table>
<thead>
<tr>
<th>GROUP B.</th>
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<th>Spleen</th>
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A. Calcium and tuberculin.
B. Calcium, cholesterol, vitamin and tuberculin.
C. Tuberculin.
## ESTIMATION OF FIBROSIS: VAN GIESON'S STAIN

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<thead>
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<table>
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<th>Spleen</th>
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</tbody>
</table>

A. Calcium, vitamin, and tuberculin.
E. Calcium, cholesterol and tuberculin.
F. Inoculated controls.
The preceding tables represent an attempt to assess the degree of fibrosis in the different groups. In the absence of complete and specific cure of tuberculous infection, healing by fibrosis is the recognised and visible manifestation of resistance triumphing over the ravages of the bacillus. It was hoped therefore that any therapeutic benefit derived from the treatments given would be revealed in an increased fibroblastic reaction. At the outset one is faced with the difficulty of forming and fixing a standard of fibrosis from which deviation can be estimated, and the result of the analysis can be but regarded as approximately accurate. A further difficulty in interpretation is the variable degree of fibrosis in different parts of the same section, and the possibility that other parts of the same organ if examined might have revealed a greater or lesser degree of fibrosis.

The lymph nodes showed the most marked and uniform fibrosis and as practically no difference was noted between them in any group they are not included in the tables for comparison.

The most striking degree of fibrosis occurred in group A - calcium and tuberculin, with group E - calcium, cholesterol, and tuberculin slightly less. Least fibrosis of any group is found in the controls.
MACROSCOPIC EVIDENCE OF FIBROSIS IN LUNGS.

TABLE XIII.

<table>
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<th>A</th>
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</table>

+ = Slight.
++ = Moderate.
+++ = Marked.

The above table will be referred to but briefly as it is difficult to estimate accurately the degree of fibrosis in an organ such as the guinea pig's lung, at post-mortem examination, without the aid of a microscope.

As estimated the greatest degree of fibrosis occurred in group D - calcium, vitamin, and tuberculin, with group E - calcium, cholesterol, and tuberculin slightly less.
**MICROSCOPIC EVIDENCE OF CALCIFICATION.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>L. Node</th>
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<td>F 3</td>
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<tr>
<td>F 4</td>
<td></td>
<td></td>
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<td>+</td>
</tr>
</tbody>
</table>

A. Calcium and tuberculin.
B. Calcium, cholesterol, vitamin and tuberculin.
C. Tuberculin.
E. Calcium, cholesterol and tuberculin.
F. Controls.
Table XIV shows the number of guinea pigs exhibiting calcification and the different organs affected. Here again where only one section was examined the results are but an approximate guide to the degree of calcification present and 'chance' is a more outstanding factor to be reckoned with in investigating this feature in the guinea pigs.

Noteworthy findings were the absence of calcification in group D - calcium, vitamin and tuberculin, and the occurrence of calcification in 5 animals in the two groups which received no calcium or any extra vitamin. De Savitch (47) found that, in rabbits treated with the combination used in group D, marked calcification and fibrosis resulted. It may be noted, however, that calcification is well known to be much more easily produced in the rabbit than in the guinea pig and this may possibly account for the discrepancy in results.

Group E is outstanding in that every guinea pig showed some evidence of calcification. In no other group did more than 3 animals show calcification. It occurred more often in the lung than in any other organ, with the spleen next in frequency. The lymphopoietic system exhibited calcification in 6 animals but as only one node was microscopically examined from each it seems probable that a more extensive examination/
examination would have revealed its more frequent occurrence in the lymph nodes. In only a few instances was the lymph node removed for section taken from the inguinal group, and, as these drained the site of primary inoculation, calcification might be expected to occur in them most frequently. The inguinal nodes were not removed for section because of the frequent occurrence of gross caseation and softening and the presence of superadded infection from the adjacent ulcer in the thigh.

An attempt was made to correlate the findings as regards the presence of calcification with the results obtained under the preceding headings. Group E—calcium, cholesterol and tuberculin, showing the most frequent presence of calcium had also the smallest average size of spleen. Of the 16 guinea pigs with microscopic calcium evident, 10 have spleens less than the average size for that of their respective groups, while 6 are above the average size.

No significant relationship was found, on comparing the duration of life of those guinea pigs with calcification, with the average longevity of their groups. Of the 16 animals with calcification 10 are below the average longevity of their group and 6 are above.

The macroscopic involvement of liver, spleen and lung/
lung in those animals with calcification showed no favourable tendency. The lesions were equally severe as in those without, and this finding is in agreement with Loewen and Oatway (40), who stated calcification was most pronounced in severe caseo-necrotic lesions. They also stated that the calcium content of the lungs of tuberculous guinea pigs is increased proportionately to the degree of tuberculosis present in the lungs but Mauer and Wells (27) disagree with this. Mauer and Wells determined the calcium content of the organs of tuberculous guinea pigs on normal and high calcium diets and they found that for most organs the rule could be laid down that the more tuberculosis present the greater the calcium content, but they excepted the lungs from this generalisation. In view of these statements it is of some interest to recall that in the present experiment calcification was found most frequently in the lungs.

Those animals exhibiting calcification when compared with the others as regards fibrosis, lymph node involvement, and the inoculation ulcer reveal no finding that merits especial attention.

In no guinea pig did 'massive' calcification occur and it may be stated that the degree of calcification in each animal showing it was approximately the same.
### CONDITION OF PRIMARY INOCULATION AT 14 WEEKS.

**TABLE XV.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Healed</th>
<th>.5 cm.</th>
<th>.5-1 cm.</th>
<th>&gt;1 cm.</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>2</td>
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<tr>
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<td>3</td>
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<td>0</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
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<tr>
<td>F</td>
<td>2</td>
<td>3</td>
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</tbody>
</table>

### CONDITION OF PRIMARY INOCULATION POST MORTEM.

**TABLE XVI.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Healed</th>
<th>.5 cm.</th>
<th>.5-1 cm.</th>
<th>&gt;1 cm.</th>
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<tr>
<td>F</td>
<td>1</td>
<td>3</td>
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<td>0</td>
</tr>
</tbody>
</table>

A
Calcium and Tuberculin.

B
Calcium, Cholesterol, Vitamin and Tuberculin.

C
Calcium and Tuberculin.

D
Calcium, Vitamin and Tuberculin.

E
Calcium, Cholesterol and Tuberculin.

F
Controls. No treatment.
Tables XV - XVI describe the condition of the site of inoculation in the left thigh and a more detailed description is given in the appendix, p. 70-71.

Four weeks after inoculation many of the guinea pigs had ulcers at the site of primary inoculation and by 6 weeks every animal showed a discharging ulcer in the left grain.

Fourteen weeks after inoculation a detailed examination of the site of inoculation was made as it was felt possible that any beneficial response to the various treatments might be manifested in the condition of the primary ulcer, and a similar detailed examination was made at the post mortem on each animal.

It is difficult to evaluate such results as it involves consideration of a factor subject to such variation as an exposed ulcer, where superadded infection is bound to play a prominent part. In addition slight variations in the site of the initial abscess would affect the subsequent course of the ulcer.

For instance some which formed close to the inguinal nodes became a discharging sinus for an underlying caseous gland mass, while other ulcers lower down the thigh with no direct glandular communication appeared to heal up fairly rapidly.
The degree of ulceration both in the course of the experiment and at death was most severe in the case of group A - calcium and tuberculin, while favourable tendencies were most conspicuous in group B - calcium, cholesterol, vitamin and tuberculin. On the whole nothing of value as to the effect of the various treatments materializes from a study of the site of inoculation.

It is commonly stated that subcutaneous inoculation of a 'non immune' animal with virulent tubercle bacilli results in a discharging ulcer that never heals. Such a statement would appear to be contradicted by the results obtained in this experiment, though it may be argued, that if death had not intervened, such ulcers as appeared completely healed would have again broken down.
E 5. Lung x 90.
Calcification, caseation, lymphocytic and epitheliod cell infiltration.
A 5. Lymphnode x 170.

Calcification of necrotic area.
E 5. Spleen x 130.

Calcification of necrotic areas, epithelioid cell infiltration..
F3. Liver x 70.

Calcification of necrotic area, epithelioid cell infiltration, and lymphocytic aggregations. Necrosis of liver parenchyma and dilatation of bile ducts.
E 5. Lymph Node. $x79$.

Illustrates calcification of necrotic areas, and tubercle follicle formation.
B 5. Spleen x 130.

Calcification of necrotic area, and tubercle follicle formation.
E 3. Lymph node. x 160. van Gieson.

Illustrates nodular type of lesion, with peripheral fibroblastic reaction tending to limit lesions.
E 3. Lymph node  x 160 van Gieson.

Nodular type of lesion with marked peripheral fibrosis.
E 3. Lung x 160 van Giesson.

Tubercle with attempted healing by fibrosis.
E 5. Lung  x 70. van Gieson.

Illustrates calcification, but less clearly than the slides stained with haematoxylin and eosin. As will be seen from this section the presence of calcium was not accompanied by any increased fibroblastic reaction.
DISCUSSION

(Including Critical Commentary on Literature).
The evidence obtained from an experiment such as this, on a limited number of animals can serve no more than as a basis for further experimentation. In the commentaries which follow the observations used to record the progress of the infection, the numerous difficulties which were encountered have already been mentioned.

Although the various factors used to judge the effect of treatment are subject to these inaccuracies it was hoped that by taking several into consideration the frequent appearance of favourable tendencies would enable more or less precise conclusions to be made. If it were possible in virulent tuberculous infection of guinea pigs to attain the therapeutic ideal of complete cure it would be unnecessary to judge the effects of treatment in this way. Unfortunately this result is not obtainable at present and it is necessary to be guarded in our interpretation of therapeutic efficacy.

It is essential that the same care be exercised in the evaluation of many of the results obtained by the different authors mentioned in the review of literature. In many instances the number of animals used in the experiments was too small to permit of conclusions being made with the assurance that some of the authors manifest. The nature of the problem makes it obvious that worthwhile conclusions can only eventuate from a large series of comparable animals.
Bogen (74), in a comprehensive analysis of the pathology of tuberculous infection in guinea pigs, and on the effects of a large series of drugs used in treatment, employed four thousand guinea pigs. These were divided into sufficiently large groups as to permit of statistical methods in the evaluation of results. Some of Bogen's conclusions will be worth referring to in considering the results obtained in the present experiment. He states "that in a large series of guinea pigs, in less than 1 per cent was the difference in the amount of tuberculosis more than half of the theoretically possible maximum deviation from the average. The amount of tuberculosis in these animals varied from zero to 16 units and half of all the deviations from the mean value will be found to fall within 1½ units above or below it." He concludes "that there is no evidence, from these data, that any perceptible difference in 'susceptibility' or resistance to tuberculosis exists between individual guinea pigs of homogenous stock raised under similar conditions and treated alike". Although he admits that an animal may deviate by as much as 9 units from the average for its age, in view of the above conclusions, any difference in results obtained in the small groups used in the present experiment may be largely disregarded as due to individual characteristics of the animals employed.
In considering the results reviewed in the literature, there are several other factors besides the inadequate size of the groups and inadequate provision of controls which prevent their satisfactory comparison. First of all much of the work has been done on rabbits and it is well known that these animals show an anomalous susceptibility to the calcifying effect of both concentrated forms of vitamin D and calcium salts. The work of Savitch et al (47) was on lines somewhat similar to those used in this research, but rabbits were used by them in coming to the confident conclusions which they did. Their illustrations revealed nodular circumscribed, fibrotic lesions in some groups, while in others they were diffuse and progressive, but, as pointed out earlier in this paper the same section from an organ frequently shows widely different lesions.

Another factor is that in much of the work different preparations of vitamin concentrates were used and not all of these were biologically standardised. Different salts of calcium have been employed and some have been administered hypodermically and others orally. In addition the varying virulence of the different types and strains of tubercle bacilli employed may influence the lesions so that comparison of results are difficult. Marked variations in the potency and effects of different types of tuberculin and the varied methods of standardisation used in its preparation will also invalidate
a strict comparison of the results obtained by its experimental use. The biochemical methods used for estimating the amount of calcium in tissues and blood serum vary considerably and widely different results are obtained in approximately the same experimental conditions. For instance, as quoted in the review of literature, Kahn & Roe (22) found 5 G. of calcium lactate administered orally to men raised the serum calcium values 30 per cent while Bauer and Ropes (23) with the same dosage reported only 8 per cent increase. When all these sources of error are taken into consideration it is not surprising to find a divergence in the results obtained by various workers.

It appears however that there is no conclusive scientific basis, as judged by experimental results, for the employment of either calcium or vitamin D in the treatment of tuberculosis, where adequate amounts of these already exist in the diet. Certainly in the present experiment the results obtained furnish no marked evidence of their therapeutic utility. At the most only favourable tendencies were noted and though most marked in the calcium, cholesterol, tuberculin, group the result cannot be upheld as convincing. The addition of vitamin D seems to have had little influence on the course of the infection or on calcification, and the group treated by the above combination with the addition
of vitamin is singularly devoid of favourable indications. The absence of calcification in the calcium, vitamin, tuberculin group, is difficult to explain on a basis of the therapeutic measures adopted, and the finding of calcification in the controls and in the tuberculin treated group suggests that in those other groups in which it is found, its occurrence is independent of the treatment. If this is so the finding is in agreement with the conclusions reached by Wells (5) that the addition of calcium to the diet is not a factor resulting in increased calcification of tuberculous lesions. Jampolis & Witt (38) stated that they did not produce calcification in tuberculous guinea pigs receiving viosterol but its occurrence here spontaneously shows that this may happen in its absence.

Fibrosis was not a dominating feature in any group in this experiment and the claims made by Savitch (47) of distinctive healing by fibrosis in rabbits could not be substantiated in the present work with guinea pigs.

The liver is said in the guinea pig to be the organ which shows the greatest degree of fibrosis in tuberculous infection. This is emphasised by Corper and Lurie (67) but it is of interest that in this experiment fibrolastic changes were not conspicuous in the liver but were most evident in the lung. It is possible that the longer duration of the infection
in the present experiment as compared with most of the experimental work on tuberculous guinea pigs accounts for the present finding.

The addition of vitamin A to the diet has had no noticeable influence on the course of the infection and this is perhaps not surprising as abundance was provided in the diet. Naturally enough, vitamin deficiency with its generally debilitating effects has an unfavourable influence on the course of tuberculosis in man and animals but this does not mean that added quantities of one or all vitamins will add to the resistance of those infected when already sufficient is supplied by a normal diet.

The assimilation of abnormal constituents in the food and of drugs from the alimentary tract of the guinea pig is a factor which may explain some of the equivocal results. The two vitamins supplied were in an oily basis and as the absorption of fat in any but very small amounts is an abnormal imposition on the metabolism of the guinea pig, it is uncertain in the present experiment to what degree the therapeutic possibilities of the two vitamins were exploited. The assimilation of calcium is affected by many dietary factors and very important is the mineral constituents of the diet. In this connection also it is uncertain if the proportions of calcium and phosphorous utilized were optimum for the guinea pig and it might have been
found that other combinations would have shown distinctive results.

Whether or not calcification in tuberculosis is a desirable event or not seems to be a debatable subject. As pointed out earlier Mavor and Wells (27) consider calcification merely a response to tissue necrosis but this view is not allowed to pass unchallenged and both Jampolis and Witt (38) and Levaditi and Po (73) concluded from their experiments that calcification was a reparative process rather than an expression of simple tissue necrosis.

No one has so far given a satisfactory demonstration of increased calcification of the lung fields of patients with pulmonary tuberculosis treated with the usual doses of vitamin D, either in concentrated form or in cod liver oil. It is therefore believed that the therapeutic benefits of cod liver oil is not due primarily to a calcification of tubercles as a result of its high vitamin D content. Calcification of primary foci of tuberculosis in humans is common and likewise in chronic adult tuberculosis extensive calcification is found in the lung fields but what particular factor is responsible for this unusual manifestation of calcification in adults is unknown. Fibrosis is the common method of tissue repair in disease and injury, and is the recognised form of healing in tuberculosis. If, by inducing calcification in tuberculous tissue we produced healing and fibrosis, then any method
which produced calcification would be a justifiable therapeutic agency. It is well recognised clinically that those relatively rare cases of extensive calcification of the lungs which occur in adult tuberculosis are of a less dangerous type and the few cases encountered by the writer have been of a chronic fibrotic nature and accompanied by very little systemic disturbance. In addition few would deny that calcification of tuberculous lymph nodes is not often a satisfactory control of tuberculous infection. Familiarity with X-ray work revealing calcified nodes in various parts of the body proves that many individuals may live to an advanced age free from any recognisable symptoms of tuberculous disease.

It is argued that, because calcification occurs in acute and progressive bovine tuberculosis without in any way influencing the course of the disease, it is not a desirable attainment in human tuberculosis. In the present work it is interesting to note that the presence of calcium appears in no way to conduce to a fibroblastic tissue reaction. The sections showing patches of calcification when stained by van Gieson's connective tissue stain reveal no greater peripheral fibrosis than occurs in lesions where there are no calcific deposits. The calcium merely seems to be deposited in certain caseous areas for no apparent reason and with no special effect on the adjacent pathological manifestations.
of the disease. On the whole the findings appear to support Well's belief that calcification occurs as a response to tissue necrosis and is of little or no reparative significance.

The use of calcium in tuberculosis on the grounds that there is a "demineralization" is not now upheld. Much of the early data on which this hypothesis rests has been valueless, for the results have been obtained mostly by analysis of the urine without consideration of either the total intake of inorganic salts or of the faecal excretion, and biochemical analysis had not yet reached its present stage of scientific accuracy. Wells (5) sums up this question "The theory that tuberculosis is accompanied by and perhaps dependent upon a "demineralization", and especially a loss of calcium from the body, has not been supported by the best controlled investigations. There has been no acceptable evidence that there is any significant decrease in the amount of calcium in either the blood or the tissues in tuberculosis. Neither has it been shown that the amount of calcium in the blood and tissues can be measurably increased, at least more than momentarily by feeding calcium compounds of whatever sort in tuberculosis or in any other condition, in which the blood calcium is not appreciably below normal."

In spite of the bulk of experimental evidence being inconclusive as regards the utility of calcium
and vitamins in tuberculosis, the weight of clinical evidence in favour of their use does not permit their hasty withdrawal from the therapeutic armamentarium of tuberculosis. The writer feels that should opportunity arise of doing similar work in the future, the methods employed in the present experiment might be elaborated and improved with perhaps more decisive findings. For one thing the necessity for larger and more numerous groups has become apparent and it may be true to say that the larger the groups the more distinctive and conclusive would the results become. A greater similarity in the ages and weights of the guinea pigs used, than in the present experiment is desirable. In order to more closely simulate the conditions of adult pulmonary tuberculosis a certain number of animals might be superinfected with virulent bacilli on a primary sensitisation of an avirulent strain. With more numerous groups the various drugs might be tried in different amounts to discover the optimum dosage. The giving of vitamin concentrates by hypodermic injection is more scientifically accurate than relying on their absorption from the alimentary tract. It is possible also that if the animals were killed at an earlier stage and post mortemed, greater differences in pathology might be manifested should the treatment have any effect on the progress of the disease. As pointed
out earlier when the animals are allowed to die of the infection the amount of tuberculosis present tends to be approximately the same. The influence of tuberculin in treatment would be more clearly demonstrated if in comparable groups it were omitted. The provision of more numerous control groups is apparent and it would be interesting to ascertain the effect of the various treatments on the weight curves and on the production of calcification in the absence of tuberculous infection.
SUMMARY.

(1) Five groups of guinea pigs, 6 in each group, were infected with tuberculosis and treated with calcium, vitamin A and D, cholesterol, and tuberculin in various combinations. A sixth group was infected but received no treatment. The various groups and their treatment were as follows:

- Group A: Calcium and tuberculin.
- " B: Calcium, cholesterol, vitamin and tuberculin.
- " C: Tuberculin.
- " D: Calcium, vitamin and tuberculin.
- " E: Calcium, cholesterol and tuberculin.
- " F: Infected controls.

(2) The influence of the various treatments on the systemic effects (S factor) of the disease was judged by the weight progress curves and the longevity. The best sustained weight curves were found in group E - calcium, cholesterol and tuberculin, and in the controls group F. The greatest gains in weight following infection occurred in group D - calcium, vitamin and tuberculin and in the group treated with tuberculin. The highest percentage loss of weight is found in the control group and next in the calcium, tuberculin group.

(3) No very striking difference in longevity was noted between the different groups. The longest survival period was found in group D - calcium, vitamin and tuberculin, with
next the control group. No one group could be said to be more susceptible to incidental infections than the others. In two groups all the animals survived to 170 days, namely group E- calcium, cholesterol and tuberculin, and group C- tuberculin only.

(4) The degree of lymph node enlargement and caseation was noted at post-mortem examination and a comparison made between the different groups. Least node enlargement was found in group C- tuberculin only, and in group E- calcium, cholesterol, and tuberculin, while greatest involvement occurred in group A- calcium and tuberculin. These tendencies were all definite but small.

(5) The amount of disease affecting liver, spleen and lung was noted in each group. The assessment of the pathological involvement revealed favourable tendencies in group A- calcium and tuberculin, with next in order group D- calcium, vitamin, and tuberculin, and group E- calcium, cholesterol and tuberculin. Group E in addition showed the smallest average splenic size though the difference in this respect from the other groups was but very slight.

(6) The type of disease revealed by microscopic examination and the degree of fibrosis could not be said to vary with the different
treatments. Evidence of sluggish spread and fibrosis was present but could not be said to clearly predominate in any one group. The greatest degree of fibrosis occurred in group D- calcium, vitamin and tuberculin, with group E- calcium, cholesterol and tuberculin somewhat less.

(7) Calcification could not be determined by macroscopic examination at post-mortem. With the exception of group D- calcium was found microscopically in all groups including the controls, and it only occurred in areas of caseation. The degree of calcification, in those animals exhibiting it, was approximately the same and in no instance did 'massive calcification' occur. Calcium was found in all four organs examined, most frequently in the lung and next in frequency the spleen. The group treated with calcium, cholesterol and tuberculin revealed calcification in every animal, and in no other group did more than three animals show calcification. It is worthy of note that, in the two groups which received neither calcium or extra vitamin in the diet, calcification occurred in five animals.

It would appear that, no therapeutic agent or group of agents as employed in this experiment could confidently be claimed to influence
influence calcification, although a tentative inference that the combination used in group E did so, might be justifiable.

(8) The inclusion of vitamin A in the diet did not appear to affect the resistance of the animals as judged by the methods used in this experiment.

(9) A critical survey of the results appearing in the literature is included in the discussion on the present research. Suggestions are offered for the carrying out of similar work in the future should opportunity arise.
CONCLUSIONS.

(1) With the therapeutic means employed in this experiment no marked prophylactic or curative results were obtained in any group as compared with the controls which received no treatment.

(2) Calcium and vitamin D did not appear to be a factor of importance in the production of calcification when given in the dosage and by the method used here. They could not be said to have influenced the amount and rate of spread of the disease nor led to any unusual degree of fibroblastic reaction.

(3) As judged by the various criteria used to assess the effect of the treatments it was found that tendencies suggesting a favourable effect were most frequently noted in the calcium, cholesterol and tuberculin group.

Calcification occurred with such frequency in this group, that, in conjunction with the other favourable effects noted, the combination may be regarded as of sufficient significance to warrant fuller investigation.

No results were obtained which would justify any of the therapeutic measures adopted in this experiment being considered applicable to the treatment of tuberculosis in humans.
APPENDIX.

POST MORTEM FINDINGS AND MICROSCOPIC DESCRIPTION OF SECTIONS.

MEASUREMENTS ARE IN CENTIMETRES.

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POST MORTEM REPORT.

A 1. Died 29.4.35.

Inoculation site is one large ulcer extending to and including inguinal region. 3 x 3 in size.

Lymphatic system: R. Groin - Caseous Gland 1.5 x .8.
3 enlarged sub-lumbar glands size .8 -.4. Firm, not obviously caseous.
No enlarged glands in mesentery.
Portal Glands mass 1.4 x 1.
Mediastinal Glands 1.6 x 1.4.
3 cervical glands enlarged - red, soft and no sign of caseation.
Axillary Glands - One enlarged and caseous left side 1.5 x .8.

Spleen: 3½ x 1½. Pale pinkish colour. Miliary grey tubercles and no caseation seen.

Liver: Moderately involved - several small caseous areas .2-.3 throughout, with larger patches 5 x 5 along the lower margin.

Lungs: Many tubercles throughout - .3-.4 in size. Caseous centres and grey firm margins. Upper and middle part of left lung is firmly adherent to anterior chest wall and to pericardium which is yellow and fibrotic at adherent area. Few fine adhesions present between R. lung and chest wall.
Kidneys: No involvement.

Lung: Tuberculous infection: small and large tubercle deposits.
   Much caseation necrosis.
   Chronic venous congestion.

Liver: Tuberculous infection: Lymphocytic infiltration and epithelioid cell reaction in portal spaces:
   Caseation necrosis: very little giant cell formation; venous congestion.

Spleen: Tuberculous infection: numerous tubercle follicles throughout the section with giant cell, epithelioid cell foci and caseation necrosis:
   General epithelioid cell reaction.

Lymph node: Extensive tuberculous infection:
   Caseation necrosis and pus formation: Replacement of lymphoid tissue by epithelioid cell reaction: Giant cell formation.

A 2. Died 6.5.35.
   Ulcer at inoculation site 1.2 x 1.

Lymphatic system: Two soft caseous glands left inguinal region size 2 x 1.5 and .9 x .6.
Sublumbar glands, soft, red. .4 x 3.
Upper/
Upper lumbar glands, small, firm .5 x .3.
Portal Glands .9 x .8. Firm.
Mesenteric Glands not involved.
Mediastinal Glands 1.8 x 1.8. Firm - caseous centres.
Cervical Glands not enlarged.
R. axilla, one enlarged gland .4 x .3.

Spleen: 6.7 x 3. Dark red in colour. No obvious caseous areas but grey mottling and streaks throughout.

Liver: Profuse involvement. Many soft caseous areas throughout. Plastic fibrinous adhesions from upper surface to diaphragm.

Lungs: Caseous tubercles .3-.4 with normal looking lung tissue between foci. Tubercles are soft and friable. No evident fibrosis. No pleural adhesions.

Heart: Moderate clear pericardial effusion.

Kidneys: No lesions.

Peritoneum: Moderate effusion. Miliary tubercles present in greater omentum.


Liver: Tuberculous infection: extensive necrosis: Small tubercle follicles throughout and little giant cell formation: Extensive congestion and serous exudation/
exudation: Atrophy of liver cells and fatty degeneration: Regeneration of bile ducts.

**Spleen:** Extensive tuberculous infection: Caseation necrosis and giant cell formation: Polymorph. leucocyte aggregations.

**Lymph node:** Tuberculous infection: Caseation and destruction of lymphoid tissue.

A 3. Died 30.5.35.

**Inoculation site** - Ulcer 1.5 x 1.

**Lymphatic system:** Left Inguinal Glands soft and caseous 2 x 1.5, .7 x .6.

Right Inguinal Glands enlarged 1.8 x .5, .6 x .3.

Sublumbar Glands - firm. .3 x 3.

Portal Gland 1 x .8.

Mesenteric Gland - caseous. 1.8 x 1.3.

Mediastinal Glands - fibro caseous. 1 x .8.

Cervical Glands - caseous. .7 x .5, .4 x .3.

**Spleen:** 7.3 x 3.8. Extensive fibro caseous tubercle.

Several large subcapsular haemorrhages present. Large adhesion from upper pole to diaphragm and ribs.

**Liver:** Profuse miliary grey tubercles with larger caseous areas .5 x .4. Congestion round most of the caseous areas.
Lung: Moderate number of tubercles .3 - .4 in size. Firm grey borders. Lung tissue between tubercles appears normal. No pleural adhesions.

Kidneys: Not involved.

Lung: Tuberculous infection: Consolidation with much proliferation of the epithelioid type of cell. Caseation necrosis. Destruction of bronchiels which show purulent catarrh. Great congestion of capillaries.

Liver: Tuberculous infection: Extensive destruction of liver parenchyma: Caseation necrosis and epithelioid cell reaction: Venous congestion: Lymphocytic and polymorph infiltration. Fatty degeneration. One or two patches of calcification present.

Spleen: Tuberculous infection: Replacement of tissue by epithelioid cell proliferation and caseation necrosis. Malpighian bodies not recognisable. Moderate amount of scattered calcium throughout section.

Lymph node: Tuberculous infection: Epithelioid cell reaction and caseation necrosis. Slight degree of calcification present.
A 4. Died 11.6.35.

Inoculation site - healed.

Lymphatic system: Left Inguinal glands soft and caseous. 2.2 x 1.3. 1.3 x 1.
Sublumbar glands firm. .4 x .3.
Upper lumbar firm, hard and fibrous, three in number. .5 x .4.

Mediastinal Glands. 1.5 x 1.2. firm, fibro-caseous.

Cervical: soft, red and not obviously involved. .4 x .3.

R. and L. Axilla - no glands enlarged.

Portal Glands. 1 x .8. Mesenteric - not enlarged.

Spleen: 4.2 x 2.2. 4 or 5 grayish yellow tubercles .3 - .5 present.

Liver: Very congested throughout. Fairly numerous caseous areas .3 x .5 in size, and a fair number of miliary grey tubercles.

Lungs: Profuse fibro-caseous lesions. Appears to be very fibrous in parts. Firm adhesions from lower lobe to chest wall. Moderate pleural effusion.

Kidneys: not involved.

Heart and Pericardium - not involved.

Liver: Tuberculous infection. Destruction of parenchyma, caseation necrosis; epithelioid and lymphocytic infiltration; congestion.

Spleen: Tuberculous infection. Epithelioid cell and lymphocytic aggregations; caseation necrosis.

Lymph node: Tuberculous infection. Caseation necrosis; giant cell and lymphocytic aggregation. Destruction of normal gland formation.

A 5. Died 19.6.35.

Inoculation site. Dry ulcer 2 x 1.

Lymphatic system: Left inguinal glands. Soft and caseous. 3 x 2 and 3 x 2.

Right inguinal Glands, caseous. 2 x 1. 1 x .8.

Sublumbar - small, firm, .4 x .4.

Upper Lumbar - small, .3 x .5.

Mesenteric - firm, fibrocaseous 2.3 x 2.

Portal - 2 x 1.5.

Mediastinal - firm fibrocaseous 2 x 2.

Cervical 2 x 1.5. .5 x .4.

Submental 1.5 x 1.5.

R.axilla. 1.6 x 1. 1 x .8. L.axilla no enlarged glands.

Spleen:/
Spleen: 5.1 x 2.3. Paler than normal. Two greyish yellow tubercles .4 x .4 and several miliary nodules.

Liver: Slight involvement. Four caseous areas .5 - .3 and a few scattered miliary tubercles.

Lungs: Profuse involvement - caseous tubercles .5 throughout and in parts caseous pneumatic consolidation. No pleural adhesions. Moderate pleural effusion.

R. Kidney: 2 small grey nodules .2 x .2.

No peritoneal effusion.


Spleen: Tuberculous infection: caseation: epithelioid cell proliferation: Malpighian bodies absent.

Two areas of calcification seen in necrotic patches.

Lymph/
Lymph node: Extensive tuberculous infection with caseation and destruction of lymphoid tissue. One or two patches of calcification are present.

A 6. Died 23.7.35.

Inoculation site. Dry ulcer 1 x .7.

Lymphatic system: Left inguinal glands soft and caseous 3.6 x 2. 3.7 x 1.9.
Right inguinal 2.2 x 1.4. .9 x 1.5.
Sublumbar - hard fibro caseous. 1.3 x 1. 1.2 x 1.
Upper lumber - firm fibro-caseous. 1.3 x 1. 1.4 x 1.
Portal 2.5 x 2.
Mesenteric - not enlarged.
Mediastinal - 1.5 x 1.5. firm.
Cervical - soft red. 1.2 x 1. 1.2 x 1.
Submental - caseous .5 x .5.
L. Axilla. 1.8 x 1.1. .7 x .6.

Spleen: 4.5 x 2.8. Heavily infected - almost completely caseous.

Liver: Slight involvement. Few caseous areas .2 - .3 in size.

Lungs: Slightest involvement seen so far. 2 or 3 caseous tubercles .4 x .4 and situated at the bases. Rest of lung appears normal. No pleural adhesions.
No peritoneal effusion.

R. & L. Kidney - normal.

Lung: Tuberculous infection: areas of follicle formation with epithelioid-giant cell systems and caseation necrosis: Intense hyperemia: Oedematous exudation into the alveoli of unaffected portion of lung.

Liver: Tuberculous infection: Extreme congestion of sinusoids and dilatation of central lobular vein: Giant cell formation and caseation necrosis.

Spleen: Extensive tuberculous infection: Obliteration of characteristic structure: epithelioid cell reaction and caseation necrosis.

Lymph node: Tuberculous infection: Small residue of lymphoid tissue: Epithelioid cell reaction and caseation necrosis.

B 1. Died 27.5.35.

Inoculation site - small ulcer - dry .2 x .2.

Lymphatic System: Left inguinal glands soft and caseous 2.2 x 1.2. 2 x 1.5.

Right inguinal - two small glands .3 x .3.

Sublumbar/
Sublumbar .5 x .4.
Upper Lumbar - firm, white and fibrous. .8 x .5. .6 x .4.
Mesenteric - two slightly enlarged. .4 x .4.
Portal Glands - 1.2 x 1.
Cervical - enlarged - caseous centres .8 x .6. .6 x .5.
Axillae - no enlarged glands.
Mediastinal 1.2 x 1.

Spleen: 5.5 x 2.3. Extensive grey, fibrous, tuberculous involvement.

Liver: Many caseous areas throughout .3 x .3. A few are larger up to .3 x .5.

Lung: Many tubercles scattered throughout lung, firm yellowish white centres with firm grey borders. Size .3 x .3. Intervening pulmonary tissue appears normal. No pleural adhesions and no effusion.

Heart: Moderate clear pericardial effusion.

Peritoneum: moderate clear effusion.


Liver: Extensive tuberculous infection: Destruction of/
of parenchyma: dilatation of bile ducts: congestion.
Tubercle follicles with caseation necrosis.
Fine granular calcification present.

Spleen: Tuberculous infection: caseation necrosis and epithelioid cell reaction: Diminution of the lymphocyte aggregations throughout the spleen.

Lymph node: Tuberculous infection: almost entirely caseous: Accompaniment of glandular tissue, salivary or pancreas.


Inoculation site - healed.

Lymphatic system:
Left inguinal glands soft and caseous. 2 x 1.1.
1 x .7. 1 x .6.
Sublumbar - firm, greyish white, .9 x .4.
Upper lumbar - small, firm. .7 x .3.
Mesenteric. Glands enlarged, firm. .8 x .6. .6 x .4.
Portal. 1.4 x 1.
Mediastinal. firm, smaller than majority 1 x 1.
Cervical. One right side, enlarged, soft and red in colour. .3 x .3.
Axillae - no gland enlargement.

Spleen: 5.5 x 3. Lateral surface practically all a caseous/
caseous necrotic mass - where not caseous it is congested and haemorrhagic.

Liver: Some large caseous areas present 2 x 1.5, 1.5 x 1. Others .5 x .5 and less. Zone of congestion around caseous areas.

Lungs: firm caseous nodules throughout .7 - .5 in size. Firm extensive fibrous adhesions from visceral pleura to chest wall and to pericardium. Moderate pleural effusion present.

Kidneys: Small greyish yellow focus near hilum. Smear - acid fast bacilli present.

Peritoneum: Moderate clear effusion.

Lung: Extensive tuberculous infection: caseation necrosis and consolidation: capillary congestion. A few small patches of calcification in caseous areas.

Liver: Tuberculous infection: Extensive caseation necrosis with destruction of liver parenchyma. Tubercle follicle formation and infiltration.

Spleen: Extensive tuberculous infection: impossible to make out any normal splenic structure.

Lymph node: Extensive tuberculous infection: almost all section is a caseous necrotic mass; polymorph aggregation.
B 3. Died 11.6.35.

Inoculation site - healed.

**Lymphatic System:**

- Left inguinal glands soft and caseous 2.2 x 1.4. 1 x .7.
- Sublumbar 1 x .6.
- Upper lumbar - firm .8 x .4. .6 x .4.
- Mesenteric - firm. 1.4 x 1.
- Portal - fibro caseous. 1 x .7. 1 x .6.
- Mediastinal - 1.5 x 1.2.
- Cervical - two enlarged soft and red - one on each side, lower cervical region. .7 x .5.
- R. axilla. 1.2 x .8. .8 x .3. firm fibro-caseous.
- L. axilla. 1.5 x .7. .6 x .3. firm fibro-caseous.

**Spleen:** 6 x 3.6. Severely involved - large caseous necrotic areas with intervening pulp very congested.

**Liver:** Numerous caseous nodules throughout .5 x .4.

**Lungs:** Extensive fibro caseous lesions .5 x .5.

Firm adhesions from lower left lung to thoracic wall.

No effusion.

**Kidneys** - Normal in appearance.

**Peritoneum** - no effusion.

**Lung:** Extensive tuberculous infection: caseation necrosis: alveolar consolidation and epithelioid cell infiltration/
infiltration: giant cell systems and polymorph aggregation.

**Liver:** Tuberculous infection: destruction of liver cells. Dilatation of bile ducts and proliferation of bile ducts. Dilatation and congestion of central lobular veins.

**Spleen:** Tuberculous infection: caseation necrosis and sinusoids infiltrated with epithelioid cells.

**Lymph node:** Extensive tuberculous infection: caseation necrosis.

**B 4. Died 20.6.35.**

Inoculation site - .5 x .5.

**Lymphatic System.**

Left inguinal glands - firm fibro-caseous 1.5 x 1.1
1.5 x 1. 2 x 1.

Right inguinal 1 x .6. 1 x .5.

Sublumbar firm 1 x .7.

Upper lumbar - firm 1 x .5.

Portal Gland mass large - firm. 5 glands varying in size from 1.5 - 1.

Mediastinal - 1.5 x 1.5.

Mesenteric - firm 1 x .8.

Cervical/
Cervical - not enlarged.
Axillary - not enlarged.

**Spleen**: 5 x 2.7. Severely involved. Upper pole caseous areas 1.5 x 1. 1.5 x 1.

**Liver**: Very slight infection - several caseous areas .2 - .4 in size. Liver not enlarged.

**Lungs**: Very slight infection - several caseous areas .2 - .4 in size. Liver not enlarged.

**Lungs**: Profuse fibro caseous involvement - some cavitation present. No pleural adhesions. Moderate pleural effusion.

**Kidneys**: appear normal.

**Peritoneum**: No effusion.

**Lung**: Extensive tuberculous infection: little alveolar structure to be discerned. Section is a mass of tuberculous tissue and caseation necrosis. Destruction and desquamation of bronchial epithelium and exudate in lumen.

**Liver**: Tuberculous infection: Extensive periportal infiltration with round cells. Miliary tubercle follicle formation with little destruction of liver parenchyma. Infection less gross than majority.

**Spleen**: /
**Spleen:** Tuberculous infection: tubercle follicle formation: giant cell and epithelioid cell infiltration. Spleen very congested.

**Lymph node:** Tuberculous infection: caseation necrosis and destruction of normal lymph cell follicles.

B 5. Died 3.7.35.

Inoculation site - healed.

**Lymphatic System:**
Left inguinal glands - soft and caseous. 2.5 x 2.
1.5 x 1.5. 1 x 1.
Sublumbar - soft caseous 1 x .5.
Upper lumbar - firm. 1.2 x .8. 1 x .8.
Portal - 1.3 x 1.2. 1 x 1. 1 x 1.
Mesenteric - not enlarged.
Mediastinal 2.5 x 2. Soft - caseous.
Cervical - two soft red glands 1.2 x .8. 1.2 x .8.
with one or two yellowish spots showing on each.
Axillae - no enlarged glands.

**Spleen:** 5.7 x 3.5. Severely involved. Caseous necrotic areas throughout. Very congested.

**Liver:** Severely involved. Many caseous areas throughout up to 1.5.

**Lungs:** Heavy infection - caseous pneumonic patches throughout. No pleural adhesions. Moderate pleural effusion.
Kidneys - appear normal.

Pericardium - slight effusion.

Peritoneum: No effusion. Miliary tubercles in greater omentum.

Lung: Tuberculous infection: fusion of tubercle follicles in parts; caseation necrosis; endothelial hyperplasia; desquamation and proliferation of bronchial epithelium and polymorphs in lumen exudate.


Spleen: Tuberculous infection: Extensive epithelioid cell reaction; disappearance of malpighian bodies; caseation necrosis. Calcification present.

Lymph node: Tuberculous infection: displacement of normal lymph follicles by tuberculous tissue; Caseation necrosis.

B 6. Died 16.7.35.

Inoculation site - Ulcer healed.

Lymphatic System:
Left inguinal glands firm fibro-caseous. 2 x 1.1, .9 x .7. 1.2 x .7.

Right/
Right inguinal - small, firm, .5 x .5.
Sublumbar - firm, 1.9 x 1.6. .7 x .6.
Upper lumbar - fibro caseous. 1.8 x 1.1. 1.9 x .9.
Cervical - not enlarged.
Axillae - not enlarged.
Mediastinal - 1.5 x 1.2.
Mesenteric - firm fibro-caseous. 1.1 x 1.8. .9 x 1.2.

Spleen: 5.8 x 3.2. Severe involvement - large caseous areas.

Liver: Not severely infected. Few small caseous cases present. .3 x .3.

Lungs: Heavily infected - large caseous pneumonic areas. Left lung adherent to chest wall. Slight pleural effusion present.

Kidneys: Small greyish yellow nodule in right kidney .2 x .2. Smear - acid post bacilli present.

Peritoneum:


Liver:/
Liver: Tuberculous infection: caseation necrosis. Periportal round cell infiltration. Necrosis and fatty degeneration of liver parenchyma.


Lymph node: Tuberculous infection: caseation necrosis and disappearance of normal node formation. Round cell and polymorph aggregation.

C 1.

The first death in this group occurred on 11.1.33, less than a month from inoculation.

At the site of inoculation there was a swelling but no rupture of the skin. Incision revealed a small subcutaneous abscess and a smear showed the presence of acid post bacilli.

One of the left inguinal glands was enlarged 1 x .7, and showed early caseation. A smear showed acid post bacilli.

There was no other sign of tuberculous involvement.

There was a moderate serous pericardial effusion but apart from that no sufficient cause of death was found.
C 2. Died 6.635.

Inoculation site - Healed.

Lymphatic System:
Left inguinal glands - firm fibro - caseous. 1 x .9.
1.1 x .9.
Sublumbar - firm, fibrous. .8 x .7.
Upper lumbar - small, firm. .5 x .3, .6 x .3.
Mesenteric - 1.6 x 1.2.
Portal - firm, 1.6 x 1.3. 1 x .6.
Mediastinal - soft, caseous. 1.5 x 1.
Cervical - two small soft red glands, .4 x .3.
Axillae - no gland enlargement.

Spleen: 4.5 x 2.6. Many miliary grey tubercles throughout with 2 or 3 larger caseous patches .3 - 1.

Liver: heavily involved. Most of left lobe is a firm caseous mass. Rest of liver has caseous patches .5 - .7 in size and also miliary grey tubercles.

Lungs: Profuse caseous pneumonia - practically no normal lung tissue except a little at anterior margins. Caseous lung is firm. No pleural adhesions. Extensive pleural effusion.

Kidneys - appear normal.

Peritoneum - moderate clear effusion.


Spleen: Tuberculous infection: disappearance of malpighian bodies: extensive epithelioid cell reaction. Necrosis. Some calcification present

Lymph node: Replacement of lymphoid tissue with tuberculous follicle tissue: Caseation necrosis and fibrosis.

C 3. Died 12.6.35.
Inoculation site - healed.

Lymphatic System:
Left inguinal glands - soft and caseous 2 x 1.5.
Right inguinal - 1.5 x 1.
Sublumbar - fibro-caseous, .5 - 1 in size.
Upper lumbar - firm. .5 - .7.
Portal - firm fibro-caseous, 1.7 x 1.3. 1.5 x 1.2.
Mediastinal/
Mediastinal - 2 x 2.
Cervical - 1.6 x 1. 1.3 x .8, fibro caseous.
Submental. Three fibro-caseous, 1 x .8, .8 x .6, .7 x .8.
Axillae - no enlarged glands.

Spleen: 5\(\frac{1}{2}\)" x 2.3. Profuse involvement - Caseous nodules throughout. Greater omentum adherent to spleen.

Liver: less involved than majority. Fair number of caseous nodules along anterior free margin and a few through rest of liver.

Lungs: Profuse fibro-caseous involvement. Nodules .5 x .5, with firm grey edges. One large caseous pneumonic patch with cavitation is present. No pleural adhesions. Moderate pleural effusion.

Kidneys: appear normal.

Peritoneum: Omentum studded with nodules .1 - .3 in size and adherent to spleen.

Blood stained peritoneal effusion.


Spleen: Tuberculous infection: tubercle follicles and caseous areas obliterating normal splenic structure. Infiltration of sinusoids with epithelioid cells.

Lymph node: Tuberculous infection: Destruction of lymphoid tissue and replacement by tubercle formation. Caseation necroses.

C 4. Died 13.6.35.

Inoculation site - Dry ulcer 2.5 x 1.5.

Lymphatic system:
Left inguinal glands - soft and caseous. 2.3 x 1.6, 1 x 1, 1 x .8.

Right inguinal - 1.6 x 1.2, .8 x .6, .6 x .4.
Sublumbar - firm, 1 x .6, .7 x .5.
Upper lumbar - firm. .7 x .5, .3 x .4.
Portal - 1.6 x 1.2, 1 x .6.
Cervical - 1.9 x 1.2. Caseous.
Submental - 1.8 x 1, 1.4 x .8, Caseous.
Mediastinal - 1.5 x 1, Caseous.

Spleen: 4.8 x 2.2. Numerous miliary grey tubercles throughout and several larger caseous areas, a5 x a5.

Liver: Numerous miliary grey tubercles with larger caseous areas, .5 - .3. Very little enlarged.

Lungs:/
Lungs: Profuse involvement. 2 or 3 large caseous pneumonic areas and several smaller caseous foci .3 - .5 in size. A few adhesions from base of R. Lung to thoracic wall are present. No pleural effusion.

Kidneys: Both have a greyish yellow nodule at the lower pole .2 x .2. Smear - acid post bacilli present.

Peritoneum - Moderate clear effusion.

Lung: Tuberculous infection: broncho pneumonic tuberculous consolidation; giant cell formation - epithelioid cell infiltration of alveoli - congestion. Calcification in caseous areas of lung.

Liver: Tuberculous infection; foci of lymphocytic aggregations in portal tracts: dilatation and proliferation of bile ducts. Venous congestion: fibrosis.

Spleen: Extensive tuberculous infection and obliteration of characteristic structure. Epithelioid cell infiltration. Caseation necrosis. A little calcification in caseous area is present.

Lymph node: Tuberculous infection. Caseation necrosis. Fibrosis.
05. Died 17.6.35.

Inoculation site - Dry ulcer 1 x 1.

Lymphatic System:
Left inguinal glands - large caseous mass 3 x 3.
Right inguinal - 1.6 x 1. Caseous.
Sublumbar - fibro-caseous 1.3 x .8, .8 x .6, .7 x .6.
Upper lumbar - .8 x .5, .7 x .4.
Portal - 1.9 x 1.4, 1 x .6. firm.
Mediastinal - fibro-caseous, 2 x 2.
Cervical - soft, red, .6 x .5, .6 x .5.

Spleen: 6 x 3. Profuse miliary involvement but no
yellow caseous areas seen.

Liver: Profuse miliary involvement - 1 - .2 in size.

Lungs: Fibro caseous lesions but not so extensive as
most. Early cavitation present. A few adhesions
from both lower lobes to thoracic wall. No
effusion.

Kidneys: Appear normal.

Peritoneum - no effusion.

Lungs: Tuberculous infection. Caseation necrosis.
Broncho-pneumonic consolidation with marked cavity
formation. Fibrosis. Several patches of calcification/
calcification present in caseous areas.


Spleen: Tuberculous infection: caseation necrosis and round celled infiltration. Marked congestion.


C 6. Died 1.7.35.

Inoculation site - 1.5 x 1.

Lymphatic system:
Left inguinal glands - caseous. 1.5 x 1.2.
Sublumbar - small, firm. .5 x .3.
Portal - small firm, .3 x .4.
Upper aortic - not enlarged.
Mediastinal - slightly enlarged, firm. .5 x .5.
Cervical - soft red with one small yellow nodule, 1 x .7, 1 x .6.
Submental - .3 x .3.

Spleen: 7 x 3.5. Miliary tubercles throughout and no/
no larger caseous areas. Very congested and soft.

**Liver**: Moderately heavy infection. A fair number of yellow necrotic areas. .3 -.4 present.

**Lungs**: Profuse fibro-caseous involvement with extensive cavitation. Fibrosis marked. Firm pleural adhesions at the bases.

**Kidneys** - appear normal.

**Pericardium** - slight effusion.

**Peritoneum** - moderate effusion.


**Liver**: Tuberculous infection: caseation necrosis and destruction of liver parenchyma. Dilatation bile ducts and proliferation of smaller bile ducts. Fibrosis. Round celled infiltration.


**Lymph node**: Tuberculous infection: caseation necrosis. Fibrosis.
Died - 2.5.35.

Inoculation Site. Ulcer 1 x .6

Lymphatic System.

Left inguinal glands. 1.8 x 1.3 1.7 x 1.4. Firm fibro caseous with soft centres.

Sublumbar. .8 x .4. .5 x .4. Firm.

Upper lumbar. .1 x .5. Firm.

Portal. 1 x .8.

Mediastinal 1.6 x 1.4.

Cervical - soft red. .4 x .3.

Spleen. 5 x 2.8. Miliary tubercles throughout and one caseous patch. .6 x .5.

Liver. Numerous caseous areas throughout. .3 - .5

Lungs. Widespread fibro-caseous tuberculous.

Firm adhesions between left lung and thoracic wall.

Adhesions from both lungs to mediastinum and pericardium.

Pericardium. Membrane is thickened and yellow fibrinous exudate is present with a thinner haemorrhagic effusion.

Kidneys. Both appear normal.

Peritoneum. Moderate - clear effusion.

Subcutaneous and connective tissue spaces are oedematosus.
Lung.
Tuberculous infection: areas of tubercle follicle formation with epitheloid giant cell systems and caseation necrosis: Intense hyperaemia and oedematous exudation into the alveoli of unaffected portion of the lung. Perivascular lymphocytic infiltration.

Liver.

Spleen.
Tuberculous infection: Diffuse areas of caseation necrosis: Giant cells: epitheliod cell reaction; Abscess formation and haemorrhage.

Lymph Node.
Tuberculous infection; Follicle formation. Caseation necrosis and fibrosis.
Died - 30.6.35.

Innoculation Site - healed.

Lymphatic System.

Left inguinal glands. Firm 1.7 x 1.3, .8 x .6
Right inguinal 1 x .6, .8 x .5.
Sublumbar. Firm fibrocaseous. .7 x .5, .8 x .6.
Upperlumbar. Firm. 1.2 x .8, 1.2 x .8.
Portal. 1 x 1, .6 x .5.
Mediastinal 1.2 x 1.

Spleen. 5 x 2.5. Caseous area .8 x .7
Otherwise it appears normal.

Liver. Profuse miliary involvement.

Lungs. Profuse fibrocaseous lesions with some cavitation present and many pleural adhesions.
No effusion.

Kidneys. Appear normal.

Lung.

Tuberculous infection; tuberculous pneumonic consolidation: Bronchial epithelium swollen and desquamated - catarrhal exudate in lumen.
Caseation necrosis. Fibrosis and cavitation.

Liver/
Liver.
Tuberculous infection; much lymphocytic infiltration and epithelioid cell reaction. Atrophy and necrosis of liver parenchyma. Marked congestion of intralobular and portal vessels. No caseation.

Spleen.

Lymph Node.
Tuberculous infection: Patches of caseation necrosis: Lymphocytic aggregations and epithelioid cell systems. Glandular tissue - probable pancreatic present.
D3.

Died - 9.7.35.

Innoculation Site. Ulcer dry. 1.9 x 1.4.

Lymphatic System.

Left inguinal glands. Soft caseous. 2 x 2.5. 1.5 x .9
Right inguinal 1.9 x .8.
Sublumbar. 1.2 x .9. 1.4 x .8.
Portal. .5 x .9. .1. x .7. 1 x .6.
Mesenteric = firm. 1.5 x .9. 1.2 x 1.1. 1.1 x 1.3.
Submental - red, soft, with small caseous firus. .8 x .5.
Left axilla. 1 x .8.

Spleen. .7 x 4 cun. Large caseous areas and numerous miliary tubercles. Omentum adhering to lower part.

Liver. Heavily infected. Many small caseous areas .2 - .4 throughout.

Lungs. Profuse caseous pulmonic tuberculosis. No pleural adhesions or effusion.

Kidneys. Appear normal.

Peritoneum. Moderate clear effusion.

Pericardium. Moderate clear effusion.

Lungs.

Tuberculous infection: fusion of fallicles - giant cell formation. Epitheliod cell and lymphocytic/
lymphocytic infiltration. Congestion of capillaries
Subpleural fibrosis.

Liver.
Tuberculous infection; epithelioid cell aggregations
Caseation and necrosis. Destruction of liver
parenchyma. Dilatation of bile ducts. Congestion
of intralobular and hepatic vessels. Haemorrhage
into portal tracts.

Spleen.
Tuberculous infection: tubercle follicle forma-
tion: Infiltration of sinusoids by epithelioid
cells and lymphocytes. Congestion haemorrhage.

Lymph Node.
Tuberculous infection; destruction of lymph
follicles; caseation necrosis and fibrosis.
Epithelioid cell infiltration.
Died 20.7.35.

Innocation Site.  Dry Ulcer.  1 x 1.5.

Lymphatic System.

Left Inguinal Glands - firm fibrocaseous.  3 x 3.
Right Inguinal - firm.  1.5 x 1.
Sublumbar - larger than in most - firm.  1.5 x 1.  .8 x .6.
Upperlumbar - large, firm.  2 x 1.5.
Portal - large fibrocaseous mass.  4 x 2.
Mediastinal - 2 x 2.
Cervical - soft red.  .8 x .6.  .8 x .8.
A#illae - no enlarged glands.

Spleen.  5 x 2.5.  Slight infection.  A few small caseous tubercles are present .2 - .3 in size.

Liver.  Moderate involvement.  Fair number of caseous areas present, .5 - .8 in size.


Peritoneum.  Slight clear effusion.

Kidneys.  Appear normal.

Lung.

Tuberculous infection.  Tubercle falcicle formation caseation necrosis.  Epitheliod cell reaction.

Bronchial/
Bronchial catarrh and exudate. Intense capillary congestion.

Liver.
Tuberculous infection; caseation necrosis. Intense vascular engorgement. Atrophy and necrosis of liver parenchyma; dilatation bile ducts; epithelioid cell reaction.

Spleen.
Tuberculous infection; disappearance of malpighian bodies and normal splenic structure. Epithelioid and lymphocytic infiltration.

Lymph Node.
Tuberculous infection; caseation necrosis.
Fibrosis.
D5.

Died - 20.7.35.

Innoculation Site - Dry ulcer. .5 x .5.

Lymphatic System.

Left inguinal glands - soft and caseous. 3 x 2.
2 x 1.5.

Right Inguinal - no enlargement.

Sublumbar - firm. 1 x .7. 1 x .7.

Upper lumbar - firm. 1.2 x .8. 1 x .8.

Mesenteric - firm mass. 2 x 2.

Portal - 2 x 1.5. 1.5 x 1.5. 1.2 x 1.2.

Mediastinal - 1 x 1.

Cervical - not enlarged.

Spleen. 4 x 2.5. Slight infection. One caseous area present. 1 x 1, and a few miliary foci.

Liver. Moderate involvement. Caseous areas .5 - 1 in size.

Lungs. Unusual appearance - very firm and cuts like firm grey cheese. Here and there are typical caseous foci. No adhesions. Slight effusion.

Kidneys - appear normal.

Peritoneum - no effusion.

Liver.

Tuberculous infection: fusion of follicles; caseation, necrosis; alveolar consolidation and infiltration epitheliod cells and lymphocytes.
Liver.
Tuberculous infection; atrophy and necrosis of liver parenchyma; lymphocytic infiltrations of portal tracts and epithelioid cell aggregations. Dilatation and proliferation of bile ducts. Haemorrhage.

Spleen.
Tuberculous infection; caseation necrosis; giant cell formation, epithelioid cell reaction.

Lymph Node.
Tuberculous infection; caseation necrosis; epithelioid and lymphocytic cellular reaction; fibrosis glandular remains, probable pancreatic.
Died - 2.8.35.

Inoculation Site - healed.

Lymphatic System.
Left inguinal glands - soft and caseous. 3 x 1.5.
Sublumbar - soft and caseous. 1.4 x 1.
Upper lumbar - firm. 1 x .6. .8 x .5.
Mesenteric Gland - firm. 1 x 1.
Portal - 1.2 x 1.2.
Mediastinal - 1.5 x 1.5.
Cervical - one enlarged - soft and red. 1 x .7.
Axillae - Right axillae. 1 x .8. .8 x .6.

Spleen. 4 x 2.2. No caseous areas. Miliary grey foci throughout.

Liver. Moderate involvement. Caseous areas throughout. .2 - .5 in size.

Lungs. Severe involvement - fibrocaseous disease with good fibrosis apparent. No adhesions.
No pleural effusion.

Kidneys - Appear normal.

Peritoneum - No effusion.

Lung:
Tuberculous infection; tuberculous broncha-pneumonia caseation necrosis; epitheliod cell reaction;
Alveolar/
Alveolar capillary congestion: Perivascular lymphocytic infiltration. Bronchial catarrh and oedema.

Liver.
Tuberculous infection; caseation necrosis; destruction of liver parenchyma; epitheliod and lymphocytic cell reaction; venous congestion; miliary follicle formation.

Spleen.
Tuberculous infection; tubercle follicle formation; caseation necrosis; epitheliod cell reaction. Vascular engorgement.

Lymph Node.
Tuberculous infection: caseation necrosis; destruction of lymph follicles; marked congestion capsular vessels. Remains of gland present - salivary or pancreas.
Died - 2.6.35.

Innoculation Site - healed.

**Lymphatic System.**

Left inguinal glands - firm fibro-caseous.  
1.8 x 1.  1.7 x .8.

Sublumbar glands - firm. .2 x .2.  .7 x .5.

Upper lumbar - firm - smaller than usual.  
.7 x .3.  .6 x .3.

Portal - 1.5 x 1.2.

Cervical - Two enlarged soft red. .4 x .3.

Axillae - none enlarged.

Mediastinum - 1.8 x 1.4.

**Spleen.** 5 x 2.6. A few small grey tubercles  
.1 - .2. No caseous areas.

**Liver.** - Numerous miliary grey tubercles throughout  
and a few caseous areas. .2 - .3, with surrounding zones of congestion.

**Lung.** Profuse fibrocaseous disease. Lung appears very congested between tubercles. No effusion.  
No pleural adhesions.

**Kidneys** - Appear normal.

**Peritoneum.** Miliary tubercles in greater omentum and slight peritoneal effusion.

**Lung.**

Tuberculous infection: consolidation: epithelioid cell proliferation: caseation necrosis: remains of/
of bronchial epithelium here and there.

Liver.
Tuberculous infection: extreme congestion of the sinusoids and dilatation of central lobular vein; giant cell formation: caseation necrosis.

Spleen.
Tuberculous infection: obliteration of the normal architecture, especially by the epitheloid cell proliferation: caseation necrosis.

Lymph Node.
Tuberculous infection: caseation and necrosis. Calcinic deposit in considerable amount.
E2.

Died 8.6.35.

Inoculation Site - Dry Ulcer 1 x .5.

Lymphatic System.

Left inguinal glands - soft, caseous, 1.4 x 1. l x .7.

Sublumbar - firm. .6 x .4.

Upper lumbar - 1 x .8.

Portal - 1.6 x 1.2.

Mediastinal - 1.6 x .6.

Cervical - two soft and red. .6 x .5. .5 x .3

Left Axilla - one caseous present. .5 x .5.

Mesenteric - .7 x .5. .5 x .4.

Spleen - 6.5 x 2.8. Many greyish yellow areas throughout. .4 - .3.

Liver. Extensive caseous area 2 cms. broad along anterior border of left lobe. A few caseous areas over remainder. .5 x .5.


Kidneys - appear normal.

Peritoneum - no effusion.

Lung.

Tuberculous infection: caseation necrosis; fusion/
fusion of caseous areas; tuberculous bronchopneumonia. Bronchiolar and alveolar catarrh and exudation: epithelioid cell proliferation. Patches of calcification present.

Liver.
Tuberculous infection; epithelioid cell aggregations and infiltration: no caseation seen. Dilatation central lobular vein and perivascular lymphacytic infiltration; Necrosis of parenchyma. Congestion and haemorrhage; Fatty degeneration.

Spleen.

Lymph Node.
Tuberculous infection: caseation necrosis. Fibrosis.
E3.

Died - 14.6.35.

Inoculation Site - Ulcer 1 x 1.

Lymphatic System.

Left inguinal glands - caseous 2.2 x 1. 1.8 x 1.
Right inguinal - .8 x .6
Sublumbar - .8 x .6. Firm.
Upper lumbar - firm. .6 x .6.
Portal - 1.6 x 1.2.
Mesenteric - One soft and red. .6 x .4.
Mediastinal - soft and caseous 1.4 x 1.2.
Cervical - two enlarged, soft and red. .7 x .5. .5 x .4.
Axillae - no enlarged glands.

Spleen. 3.8 x 2.3. Several large caseous areas showing less distinctly than in most. Spleen considerably smaller than most seen.

Liver. Profuse involvement - many caseous tubercles throughout. .1 - .4 in size.

Lungs. Numerous caseous foci throughout .1 - .4 in size. Parenchyma between lesions appears normal. No adhesions. Moderate pleural effusion.

Kidneys - appear normal.

Peritoneum - no effusion.
Lung.

Liver.

Spleen.
Tuberculous infection: caseation necrosis throughout most of section obliterating normal architecture. Marked congestion.

Lymph Node.
Tuberculous infection; caseation necrosis: fibrosis. Calcification present.
E4.

Died - 17.6.35.

Innoculation Site - dry ulcer .7 x .4.

**Lymphatic System.**

Left inguinal glands - 2 soft and caseous and 2 firmer. 1.3 x 1, 1.5 x 1, 1 x 1, 1.3 x 1.

Right inguinal. .5 x .5.

Sublumbar - firm. .5 x .3, .5 x .3.

Upper lumbar - .7 x .4, .6 x .3.

Portal. 1 x .8.

Mediastinal - 1 x .8.

Cervical One left side caseous 1.1 x .7.

**Spleen.** 5.3 x 2.5. Extensive infection. Caseous lesions throughout .2 - .5 in size.

**Liver.** Several caseous areas. .5 - .6 and others .1 - .7. throughout.

**Lung.** Profuse involvement. Lesions are not the yellow caseous type usually seen but grey and are noticeably tough to cut. Extensive cavity formation is present. Pleural adhesions from lower right lung to chest wall. No effusion.

**Kidneys.** Appear normal.

**Peritoneum.** No effusion.

**Lung.**

Tuberculous infection: consolidation: epitheliod cell proliferation: caseation necrosis: bronchial catarrh/
catarrh. Intense capillary engorgement. Small patches of calcification present.

Liver.
Tuberculous infection: caseation necrosis destruction of liver parenchyma; dilatation of bile ducts and proliferation of smaller ducts; Very congested: capillary haemorrhage.

Spleen.
Tuberculous infection: numerous caseous foci throughout; epithelioid cell proliferation: destruction of normal structure: glandular tissue present, probably pancreas.

Gland.
Tuberculous infection: caseation necrosis.

Fibrosis.
Patches of calcification present.
Died 23/6/35.

Innoculation site - .5 x .5.

**Lymphatic System.**

Left inguinal glands - soft, caseous 2 x 1.5, 1.5 x 1.
Right inguinal - .5 x .5
Sublumbar - firm. 1.3 x .8. 1 x .8.
Upper lumbar - 1.4 x .7, 1.2 x .6.
Portal - 1.5 x 1, 1.7 x 1.2. 1 x .7.
Axillae - left side. 1 x .5 caseous.
Cervical - soft, red. .6 x .4, .4 x .3.
Mediastinal - 2 x 2.

**Spleen.** 6 x 3. Heavy involvement. Caseous areas throughout. .7 x .5 in size.

**Liver.** Moderate involvement - Caseous areas throughout. .5 and less in size.

**Lung.** Profuse fibrocaseous involvement with caseous pneumonic patches. No pleural adhesions. Slight pleural effusion.

**Kidneys** - appear normal.

**Peritoneum** - no effusion.

**Lung.**

Tuberculous infection. Caseation necrosis epithelial cell proliferation: alveolar consolidation: emphysema: Perivascular lymphocytic infiltration; congestion.

**Calcification present.**

Liver/
Liver.

Spleen.
Tuberculous infection: disappearance of malpighian bodies: epithelioid cell aggregations and infiltration of sinusoids, numerous caseous foci present. Calcification present.

Lymph Node.
Tuberculous infection: caseation necrosis: much fibrosis. Patches of calcification present.
Died - 1.7.35.

Inoculation Site - Ulcer 1 x 1.

**Lymphatic System.**

Left inguinal glands - caseous. 2.2 x 1.5. 1.5 x 1.

Right inguinal - caseous. 1.5 x 1.

Sublumbar - 1 x .7. 1 x .7

Upper lumbar - 1 x .5. 1 x .5.

Portal. - 1.5 x 1.3.

Mesenteric - firm. 1.7 x 1.3.

 Mediastinal - 1.5 x 1.4.

Cervical - one caseous. 1 x .7; another soft and red. .7 x .5.

Axillae - left. .8 x .5.

**Spleen** - small 4.5 x 2. No tubercle seen.

**Liver** - very slight involvement. No sign of infection except lateral part of right lobe where is a caseous area. 1 x 1.

**Lungs.** - slight involvement comparatively. A few firm grey tubercles .1 - .2 in size. Lungs small and consolidated, probably due to the marked pleural effusion which is present. No adhesions.

**Kidneys** - appear normal.

**Peritoneum** - no effusion.

Lung/
Lung.

Liver.
Tuberculous infection: caseation and necrosis: several lymphocytic foci throughout; congestion: great dilatation of bile ducts.

Calcification present.

Spleen.
Tuberculous infection: epitheliod cell proliferation and infiltration: some areas of caseation necrosis.

Lymph Node.
Tuberculous infection: caseation necrosis: disappearance of lymph follicles and fibrosis.
Died - 14.5.35.

Inoculation Site - Sinus 0.3 x 0.3.

Lymphatic System.

Left Inguinal Glands - soft and caseous. 1.5 x 1, 1.5 x 0.5.

Sublumbar - firm. 1 x 0.6, 0.7 x 0.5.

Upper lumbar - 0.8 x 0.6, 0.7 x 0.5.

Portal - small. 0.5 - 0.1.

Cervical - no enlargement.

Axillae - no enlargement.

Mediastinal - 1.8 x 1.6.

Spleen. 5 x 2.5. Not markedly affected N.E.

A few small grey areas present. 0.1 - 0.2 in size.

Liver. - Relatively slight involvement. A few small caseous areas 0.2 and less in size present.

Lung. Profuse involvement. Part of lobes show caseous pneumonic consolidation and between these patches are caseous foci 0.4 - 0.3 in size. No adhesions. No effusion.

Kidneys - appear normal.

Peritoneum. - slight effusion. Omentum studded with grey tubercles 0.2 - 0.3 in size.

Lung.

Tuberculous infection: marked congestion of vessels; oedema/

Liver.
Tuberculous infection: marked venous congestion: Dilatation of central lobular vein and of sinusoids; atrophy of parenchyma. Tubercle follicle formation; epithelioid cell reaction: polymorph infiltration: no giant cells; commencing caseation. Perivascular lymphocytic infiltration.

Spleen.
Extensive tuberculous infection: obliteration of characteristic structure: epithelioid cell reaction and caseation necrosis. Infiltration with inflammatory cells - plasma cells and polymorphs: haemorrhage.

Lymph Node.
Tuberculous infection: epithelioid cell reaction: caseation necrosis.
F2.
Died - 2.6.35.
Innoculation Site - ulcer .2 x .2.

Lymphatic System.
Left Inguinal glands - one large caseous abscess 4 x 2.
Sublumbar - .3 x .3.
Upper lumbar - .5 x .3, .4 x .3.
Portal - 2 x 1.
Mesenteric - firm 1.2 x .8, .6 x .6
Mediastinal - 1.6 x 1.6.
Cervical - M. side 1.7 x 1.2, .7 x .7.
Submental 1 x .3, .6 x .6

Spleen. 6 x 3.5. Profuse involvement. Two large caseous areas 1.5 x 1.5, and many other small caseous foci.


Lungs. Widespread fibrocaseous disease. Foci .5 x .5 in size with caseous pneumonia in parts. No pleural adhesions. Moderate pleural effusion.

Kidneys - appear normal.

Peritoneum - moderate clear exudate.

Lung.
Tuberculous infection: Tubercle follicles of typical appearance: Extreme capillary congestion of the residual lung tissue; epitheliod cell reaction; remains of bronchi.
Liver/
Liver.
Tuberculous infection: scattered tubercle follicles with epitheliod cell - giant cell architecture; lymphocytic infiltration: focal necrosis of liver parenchyma.

Spleen.
Tuberculous infection: extensive caseation necrosis; giant cell formation: a generalised epitheliod cell proliferation with disappearance of malpighian bodies.

Lymph Node.
Tuberculous infection: a few tubercle follicles in lymphoid tissue; caseation necrosis: giant cells: fibrosis.
F3.

Died - 23.6.35.

Innoculation Site - Dry Ulcer .5 x .4

Lymphatic System.

Left inguinal glands - soft, caseous, 2 x 1.5, 1 x .8.
Right inguinal - 1 x .7.
Sublumbar - firm. 1 x .7.
Upper lumbar - 1 x .7, .7 x .5.
Mesenteric - .6 x .5.
Portal - 1 x .8.
Mediastinal - 2 x 2.
Cervical - one caseous 1 x .5. One soft, red, .7 x .5.

Spleen. 5.5 x 3. Profuse involvement. Many caseous areas throughout .5 and less in size.

Liver. Moderately severe infection. Caseous areas throughout 14 x .3 in size.

Lungs. Profuse fibrocaseous disease. Lesions dark grey in colour and feel firm and fibrous. Firm adhesions from middle lower lung to chest wall and diaphragm. No effusion.

Kidneys - Appear normal.

Peritoneum - No effusion.

Lung/
Lung.
Fairly extensive calcification.

Liver.
Tuberculous infection: caseation necrosis: focal atrophy and degeneration of parenchyma. Lymphocytic cell aggregations and focal tubercle formation and epitheliod cell infiltrations. Dilatation and proliferation of bile ducts: dilation of central lobular vein and sinusoids.
Calcification present.

Spleen.
Tuberculous infection: many caseous foci throughout section: lymphocytic aggregations: epitheliod cell infiltration.

Lymph Node.
Tuberculous infection: caseation necrosis: epitheliod cell reaction: fibrosis.
F4.

Died 10.7.35.

Innoculation Site - Healed Ulcer.

Lymphatic System.

Left Inguinal glands - soft caseous. 2.2 x 1.8. 2 x 1.

Right Inguinal. .5 x .5. .4 x .4.

Sublumbar - firm. 1.2 x .8. .6 x 1.

Upper lumbar - .8 x .6. .6 x .5

Mediastinal - 1.4 x 1.

Portal - 1.2 x 1.

Cervical - not enlarged.

Spleen. 5.2 x 2.5. Profuse involvement - many large caseous areas throughout.

Liver. Moderate involvement - several caseous areas .6 - .4 in size, with a few small grey foci.

Lungs. Profuse involvement - fibrocaseous disease with caseous pneumonic patches. Firm adhesions to thoracic wall. Moderate effusion.

Kidneys. Small grey nodule left kidney. .2 x .2.

Smear acid fast bacilli present.

Peritoneum - no effusion.

Lung.

Tuberculous infection: alveolar consolidation and infiltration with epithelioid cells: caseation necrosis/
capillary congestion: endarteritis; fibrosis.

Liver.
Tuberculous infection: caseation necrosis; intense engorgement of central lobular vein and sinusoids; haemorrhage: atrophy and degeneration of parenchymal cells: epithelioid cell systems and perivascular lymphocytic infiltration.

Spleen.
Tuberculous infection: tubercle follicle formation and fusion; epithelioid cell infiltration and obliteration of normal splenic structure.
Calcification present.

Lymph Node.
Tuberculous infection: caseation necrosis: destruction of lymphoid follicles: fibrosis.
F5.

Died 12.8.35.

Innoculation Site - dry ulcer .6 x .5.

Lymphatic System.

Left inguinal glands - soft caseous 2.4 x 1.5.
Right inguinal - 1 x 1. .6 x .9. .5 x .5
Sublumbar - 1 x .8
Upper lumbar - 1 x .6.

Mesenteric - fibrocaseous mass. 2 x 1.7.

Mediastinal - 2 x 1.8.

Cervical - firm, caseous .6 x .6.

Portal - 1.8 x 1.2.

Spleen. 5.5 x 3.

Extensive involvement. Miliary grey tubercles throughout.

Liver. Moderate involvement. Left lobe mainly affected with miliary grey tubercles.

Lungs. Extensive fibro-caseous involvement most marked in upper lobes. Moderate pleural effusion. No adhesions.

Kidneys - appear normal.

Peritoneum - no effusion.

Lung.

Tuberculous infection: tubercle follicle formation and/
and fusion: alveolar consolidation and epitheliod cell infiltration: lymphocytic aggregations present; extreme capillary engorgement: emphysema.

Liver.
Tuberculous infection: a few foci of tubercle formation with some caseation necrosis: little epitheliod cell formative: congestion of vessels.

Spleen.
Tuberculous infection: caseation necrosis and obliteration of normal structure and malpighion bodies: congestion and haemorrhage.

Lymph Node.
Tuberculous infection: caseation necrosis: epitheliod cell systems and lymphocytic infiltration: fibrosis.
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CONDITION of PRIMARY INOCULATION on 26.3.35.

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<thead>
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<th>Group A</th>
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<td>(1) 1.5 x .4 almost dry.</td>
<td>(1) Healed.</td>
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<tr>
<td>(2) .6 x .1 dry ulcer.</td>
<td>(2) .3 x .4</td>
</tr>
<tr>
<td>(3) .2 x .2 2 sinuses.</td>
<td>(3) Healed.</td>
</tr>
<tr>
<td>(4) 3 x 2 x 1. large crater. (4) .2 x .2 sinus.</td>
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<tr>
<td>(5) .4 x 3 dry ulcer.</td>
<td>(5) .6 x .4 almost dry.</td>
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<tr>
<td>(6) .1 x .1 sinus.</td>
<td>(6) Healed.</td>
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<table>
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<th>Group C</th>
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<tbody>
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<td>(1) Healed.</td>
<td>(1) .4 x .2.</td>
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<tr>
<td>(2) Healed.</td>
<td>(2) .7 x .4, dry ulcer.</td>
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<tr>
<td>(3) .3 x .2 sinus</td>
<td>(3) .2 x .2 sinus</td>
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<tr>
<td>(4) .2 x .2 dry ulcer.</td>
<td>(4) Healed.</td>
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<td>(5) .6 x .5 dry ulcer.</td>
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<table>
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<th>Group F</th>
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<tbody>
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<td>(1) .5 x .5, dry ulcer.</td>
<td>(1) Healed.</td>
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<tr>
<td>(2) Healed.</td>
<td>(2) Healed.</td>
</tr>
<tr>
<td>(3) 1 x .6</td>
<td>(3) .4 x .2, sinus.</td>
</tr>
<tr>
<td>(4) .4 x .4</td>
<td>(4) .2 x .2, sinus.</td>
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<tr>
<td>(5) .4 x .4 dry ulcer.</td>
<td>(5) .2 x .2, dry ulcer.</td>
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<td>(6) .4 x .4</td>
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### CONDITION of PRIMARY INOCULATION at POST MORTEM.

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<th>Group A</th>
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<td>(1) 3 x 3 cms.</td>
<td>(1) .2 x .2, dry ulcer.</td>
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<tr>
<td>(2) 1.2 x 1 &quot;</td>
<td>(2) Healed.</td>
</tr>
<tr>
<td>(3) 1.5 x 1 &quot;</td>
<td>(3) Healed.</td>
</tr>
<tr>
<td>(4) Healed.</td>
<td>(4) .5 x .5.</td>
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<tr>
<td>(5) 2 x 1, dry ulcer.</td>
<td>(5) Healed.</td>
</tr>
<tr>
<td>(6) 1 x .7, dry ulcer.</td>
<td>(6) Healed.</td>
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<table>
<thead>
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<th>Group C</th>
<th>Group D</th>
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<td>(2) Healed.</td>
<td>(2) Healed.</td>
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<tr>
<td>(3) 2.5 x 1.5, dry ulcer.</td>
<td>(3) 1.8 x 1.4, dry ulcer.</td>
</tr>
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<td>(4) 1 x 1, dry ulcer.</td>
<td>(4) 1.5 x 1, dry ulcer.</td>
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<td>(5) 1.5 x 1.</td>
<td>(5) .5 x .5, dry ulcer.</td>
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<td>(1) Healed.</td>
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<td>(2) 1 x .5, dry ulcer.</td>
<td>(2) .2 x .2</td>
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<td>(6) 1 x 1.</td>
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REFERENCES.

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