Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 19, *Numbers* 1 *and* 2, *January and April,* 1944.

Printed in the Union of South Africa by the
Government Printer, Pretoria.

The Tuberculin Test in Guinea-Pigs and Cattle. The Allergic Response of Animals to Tuberculin and to Extracts of Non-Pathogenic Acid-Fast Bacteria.

By A. S. CANHAM, Officer in Charge, Veterinary Research Laboratory, Allerton, Pietermaritzburg, Natal.

In 1882 Koch announced the discovery of the organism causing tuberculosis. As a result of further work by him he observed that a certain reaction was obtained from guinea-pigs suffering from tuberculosis when they were injected with live organisms of this disease. This reaction, which became known as Koch's phenomenon was not obtained when healthy guinea-pigs were inoculated with virulent organisms of tuberculosis. This same reaction was again given when tuberculous guinea-pigs were inoculated with dead organisms of tuberculosis. The conclusion arrived at as the result of this work was that the reaction was set up by some substance or substances common to both dead and live organisms of tuberculosis.

Koch now desired to obtain this active principle in solution and by growing *M. tuberculosis* in glycerine broth for 6 to 8 weeks at 38° C., killing the growth at 100° C., concentrating it down to $^1/_{10}$ of its volume and finally filtering it, he was able to prepare a product that was, and still is, known as Koch's old or original tuberculin. The reactions given to this tuberculin made Koch (1890 and 1891) hopeful of using this product both as a prophylactic agent and a curative agent for tuberculosis. Unfortunately these expectations were only realised to a very slight degree.

Other workers such as Guttman, Roeckl and Schuetz, Bang and Salomonsen, Lydtin, Bang, Nocard, Hutyra and Calmette investigated the possibilities of using tuberculin as a diagnostic agent for the presence of tuberculosis in cattle with encouraging results.

In 1892 Nocard formulated a method whereby the tuberculin should be used and described how the resulting reactions should be interpreted. This may be regarded as the commencement of the use of tuberculin as a diagnostic agent for the presence of tuberculosis. This test was known as the subcutaneous test on account of the tuberculin being injected under the skin of the subject to be tested. All this work was carried out on cattle.

Tuberculin Tests.

Following closely on the adoption of the subcutaneous test for the diagnosis of tuberculosis, many workers further investigated the use of

tuberculin in an attempt to improve and find new methods of testing. Van Pirquet (1907) introduced the cutaneous test which consisted of three scarifications of the skin, 3 to 4 mm. long at intervals of a few centimeters. On two of these scarifications was placed and spread a drop of Koch's raw tuberculin diluted 1–4 in sterile glycerine. The third scarification served as a control and received no tuberculin. The individual on whom the test was carried out was examined after 24 and 48 hours. (Calmette.) This method was utilized by Vallée in cattle. Some reliable results were obtained, but the general view held was that it was unreliable and, therefore did not find favour with the owners of the tested cattle. Slight modifications of van Pirquet's test were made. Lignières (1908) simply shaved the skin and rubbed in a few drops of raw tuberculin without having first scarified the part treated. This test has not been carried out extensively on cattle. Moro (1908) incorporated raw tuberculin in lanoline and rubbed the ointment over some small tender area of the body. This has also been tried on cattle. Malou (1932) reports the use of special discs of small pieces of paper soaked in concentrated tuberculin and applied to the skin without prior scarification. He claimed reliable results for this method. Wolff-Eisner and Calmette (1907) described reactions obtained by instilling one drop of dilute tuberculin into the conjunctival sac of tuberculous human beings. Vallée tested this out in tuberculous animals the same year with promising results. More reliable results are obtained by instilling concentrated solutions of tuberculin into the eye. This test did not find general favour on account of its unreliability. It is known as the ophthalmic test.

Mantoux (1908) introduced the injection of a dilute solution of tuberculin into the dermis as a test. Moussu injected concentrated tuberculin into the dermis of the eyelids of cattle, and this became known in veterinary circles as the intradermopalpebral test. A further modification of this test, the intrapalpebral, was introduced by Favero and Finzi (Hutyra, Marek and Manninger). They injected the tuberculin into the subcutaneous tissues of the eyelid and the results given combine to an extent the reactions following the subcutaneous, intradermal and even the ophthalmic tests. Finally, Schmidt and Haupt and Januschke (Hutyra, Marek and Manninger) injected concentrated tuberculin into the connective tissue of the conjunctiva—this is known as the subconjunctival test. The reactions obtained by this method resemble those seen in the intrapalpebral test. It has been claimed that these last two methods of testing yield even more reliable results than those given by the subcutaneous test.

The intradermal test proper was carried out first by Moussu and Mantoux (1932) and achieved great popularity. After having tried this method out extensively in Great Britain, a report was published by Buxton and his co-workers (1925) and presented to the British Tuberculin Committee of the Medical Research Council. As a result of experience, a modification was suggested in the method of application of the test. Instead of a single injection intradermally, two intradermal injections into the same site were to be made. This test is known now as the double intradermal and is preferred to the subcutaneous test although at times they are used in conjunction.

Tuberculin Testing in South Africa.—Up to 1928 the subcutaneous test was the method in vogue for the diagnosis of tuberculosis in cattle and apparently gave good results (Chalmers, 1927). There were, however, a number of objections raised to its continued use by Viljoen (1927) and Power (1927). This test could be applied to tame stabled cattle, but when it came

to the testing of semi-wild or ranch cattle, its results could not be relied upon with any degree of certainty (Goodall, 1927). The mere fact of bringing such animals into a stable and the handling was often followed by a rise in temperature which obscured the interpretation of the test. The long periods of observation and the frequent taking of temperatures were other objections raised to this method of testing. With the introduction of the double intradermal method, the taking of temperatures was done away with as a routine measure as the reaction, if any, was usually chiefly local. The result was that any animal whether accustomed to being handled or not, stabled or running wild, could be tested successfully. As is recognised, however, neither this nor any biological test is 100 per cent. reliable.

The Intradermal Test.—In most parts of the world this is now the recognised test for the presence of tuberculosis in animals. Some countries use the single method, i.e., one injection of tuberculin only, while others have adopted the double method, i.e., two separate injections of tuberculin into the same site in the skin at an interval of 48 hours. Each method has its own advocates. In the experience of the writer a positive reaction will usually be shown after 24 hours after the first intradermal injection. Moussu and Mantoux (1932) recommended the caudal fold for the site of the injection, while others prefer the injection to be made into the skin at the side of the neck. In South Africa the subcaudal fold was at first the site used, but owing to this area being one of the predilection sites for tick infestation this was given up in preference to the side of the neck. This site is also used in Great Britain.

Difficulties of the Test.—Certain objections have been raised to this method of testing. It is claimed firstly that a number of animals that give positive reactions do not show lesions of tuberculosis in spite of a careful post-mortem examination, and secondly a number of animals give reactions which are neither definitely positive nor definitely negative, thus making it difficult for the operator to give a correct decision.

It is not the intention of the writer to consider fully the different suggested explanations for these two problems, but brief mention will be made about each.

Technique.—Certain errors in carrying out the test may give rise to difficulties in interpreting results. Buxton and MacNalty (1928) stress the fact that the point of the needle should lie in the deepest layers of the derma. If by chance the needle enters the subcutaneous tissues no local reaction will result. On the other hand, if the injection is made immediately under the surface epithelium, swellings will occur which are difficult to interpret. In tuberculous animals such an injection may give rise to a hard circumscribed swelling with but little oedema (Buxton and MacNalty, 1928). Wier (1935) states that superficial injections may cause in reactors a large but circumscribed painful nodule which is unattached to the underlying skin, but it may also occur in hide-bound animals. In discussing injections made too deep, he also asserts that swellings may reach 25 mm. in diameter but be hard and circumscribed or the tuberculin may spread giving rise to an elongated swelling with ill-defined edges. In neither case is the swelling accompanied by pain. De Kock (1932) mentions "the delicate operation of introducing the tuberculin at the proper part". Green (1939) draws attention to the site of injection. He stresses the importance of not injecting too low down on the neck in case the oedema in a reactor may be masked by

loose folds of skin. He also states that in this situation the oedema may disappear more rapidly than it would do if the injection had been made higher up the side of the neck where the skin is fairly firm and tense.

Finally in bulls and oxen with extremely thick skin over the neck region the injection should be made over the shoulder blade, where the skin is usually thinnest. Washing of the site of injection is recommended by Buxton and MacNalty (1928) while Wier (1935) and Douglas (1937) state that disinfection of it is contra-indicated. The writer in carrying out tests of many animals in the Union has never disinfected the skin.

Jorgenson (1937) states that when tuberculin is injected into the deeper layers of the skin a stronger reaction develops than when it is injected more superficially. He further discusses the intensity of reactions in the intradermal test as used in Denmark. An analysis of 85 animals' reactions was 0·22 mm. greater when the injection was made behind the shoulder instead of in front of it. He experimented with varying doses of tuberculin (0·05, 0·1, and 0·2 c.c.) for injection and showed that the largest dose caused the strongest reaction but stated that the smallest dose gave an unmistakable one. Buxton and MacNalty (1928) recommend a dose of 0·1 c.c. Buxton and Glover (1939) stress the fact that concentrated tuberculin in doses of 0·1 c.c. should be used for cattle owing to the low order of dermal sensitivity in these animals.

Sâ Viana Conte. H. and Belo, M.M.A. (1936) state that in Portugal the intradermal tuberculin test has given much better results when performed on the side of the neck than in the caudal fold.

Various opinions are expressed about the measurement of the skin during testing. Tutt (1928) in a personal communication to a questionnaire sent out by Buxton and MacNalty (1928) suggests the discontinuance of measurements in favour of palpation. These two workers could not agree entirely with him, but state that the true criteria of a positive reaction are the production of swelling, heat and tenderness around the site of injection while the measurement of the increase in skin fold thickness is simply a method of recording the degree of swelling produced by the injection. They give figures for average skin measurement increase in 39 non-tuberculous animals as 3 mm. and for 39 tuberculous animals as 12·74 mm. Finally they state that it is impossible to lay down any definite increase in skin measurement which could be taken as absolutely diagnostic of tuberculosis.

Glover (1931) states that the character of the swelling is of greater importance than the size. Green (1933) as a result of his experiences in carrying out the tuberculin test in Durban, comes to the conclusion that it is better to rely on the clinical character of the swelling than merely on the increase of skin measurement. He goes so far as to state that after some experience it is better to discard the callipers altogether. Laenkolm (1934) after discussing the Danish regulations for the testing of cattle, states that in his opinion the palpation of the swelling is generally the most reliable measure for deciding a positive or negative reaction. Wier (1935) holds the view that measuring of the skin is not to be recommended. Douglas (1937) does not advise measuring the skin at the time of injection, but at the 48th hour he measures a normal skin fold and then the site of the swelling resulting from the first injection.

On the other hand very definite views are held by Continental authorities as to the essential part played by the skin measurements in assisting the interpretation of a test.

Stenius (1932) points out the bad points of the slide rule callipers commonly used for measuring the skin. The disadvantages claimed are that they are difficult to manipulate accurately with one hand and the minimum unit of the scale, viz., the mm., is considered too large. He introduced an improvised instrument which shows the readings up to a tenth of a millimetre.

Christiansen (1933) states that callipers must be accurate and graduated in half-millimetres. In his opinion, in a herd believed to be free from tuberculosis where the single intradermal test has been carried out, an increase in measurement of the site of injection of 2·5 mm. or over is indicative of a positive reaction. In other herds an increase of 3·5 mm. or over in the skin measurement is classed as a positive reaction while an increase of between 1·5 mm. and 3·5 mm. suggests a suspicious case.

Götze and Muller (1936) describe a special form of callipers in which a spring regulates the compression of the jaws of the instrument in order to obtain a fixed and constant pressure in all measurements of the skin. A geared pointer on a dial gives measurements within 1 mm.

Zeller (1936) decided that an increase of 3 mm. in the skin measurements indicates a positive reactor and states that on post-mortem examination 96·8 per cent. of these reactors confirmed his views. It must, however, be stated that this work was carried out on animals that had already been condemned for open tuberculosis by clinical examination.

Hancock (1939) gives some views on what he terms a " Pinch " and a " Wipe " measurement and shows that there may be a difference of 4 mm. or more in one swelling by these two methods. The possibility of obtaining such differences was due to his using callipers of the compass type, i.e., two arms with a ball on each end instead of the usual sliding bar type commonly in use. He is in favour of the " pinch " measurement being adopted for interpreting the degree of swelling. Llewellyn Jones (1939) criticises Hancock on his suggestions regarding " pinch and wipe " measurements and advises against using the compass type of calliper, prefering the usual sliding bar type.

With these different methods of technique and different rules laid down by various operators, it is quite conceivable that errors in interpretation may take place.

When the interpretation of reactions is considered, various opinions are again expressed. Malcolm (1932) discusses the value of tuberculin tests and states that apart from human errors or unsuitable tuberculin, the test is 100 per cent. accurate. The Australian workers have marked views on the interpretations of tests. Henry (1936) states that the disadvantages of the intradermal test are that results and the interpretation of the test rest so much on the skill and ability of the operator. Fethers, cited by Symonds (1936) points out that " no lesion " reactors are often due to the testing being done under difficult circumstances and to the post-mortem search for lesions not being sufficiently thorough.

In a discussion at a meeting of the Lancashire Division of the National Association of Veterinary Medical Associations in December, 1932, it was agreed in the main that the demonstration of heat and pain is necessary for the diagnosis of a positive reaction as well as local swelling.

Spicer (1932) lays stress upon the importance of recognising slight oedema in reactions. Green (1933) is of the opinion that a definite positive or negative reaction is easy to interpret and holds that the cardinal symptoms of a positive reaction are the increase in skin measurements accompanied by oedema, pain and heat, while confusion only arises in interpreting results where border-line or doubtful reactions are experienced. He classifies these reactions as—

1. Slight oedema with no pain on palpation.
2. Slight oedema with pain on palpation.
3. Hard oedematous swellings.

He advises operators not to give a definite opinion if they encounter reactions of this nature but to retest such animals within a fortnight. His descriptions of each of these groups are of much use to those who have to conduct many tuberculin tests on cattle.

Wier (1935) asserts that enlargement of the site of injection accompanied by slight pain may be caused where rubbing, due to irritation, takes place. He is of the opinion, however, that reactors do not rub the site owing to pain. In interpreting results, he states that reactions should not be judged by the size of the swelling but by its nature and holds strongly to the view that the cardinal symptom of a reaction is the presence of pain.

Douglas (1937) has different views regarding pain accompanying reactions of any nature and expresses the view that little weight should be given to the presence of pain as a deciding factor in the case of a doubtful reactor. He mentions other symptoms which may assist one in coming to a definite conclusion, such as enlargement of the prescapular gland on the side injected, systemic symptoms such as rigors, a high temperature, and a drop in the milk yield. The writer has also experienced cording of the lymphatic vessels close to the site of injection in some positive reacting animals. In conclusion Douglas holds that the character of the swelling is of much greater importance than its size.

Sensitization of Cattle to Tuberculin.

In attempts to explain " no lesion " reactors, many explanations have been suggested. Schroeder (1926) expresses the view that undue importance is given to the question of the " no lesion " reactor, holding that the number of such reactors is negligible in comparison with the number of animals tested. He says, " Tuberculosis in most instances is a chronic slowly progressive disease and the tuberculin test supplies no information about the age, the volume of the lesions nor the virulence of the responsible tubercle bacilli. Bearing this in mind we cannot fail to appreciate that it would be surprising if we did not find some animals that react before lesions can be discovered in their bodies." This is the " presensitive stage " referred to by de Kock (1932). Schroeder goes further and states that the Federated Bureau of Animal Industries of the United States of America examined tissues from several thousand so-called " no lesion " reactors and proved without doubt that about 25 per cent. were infected with tubercle bacilli or were early, incipient, or undeveloped cases of tuberculosis.

Moussu (1932) suggests that cattle in heavily infected herds may inhale large numbers of desiccated tubercle bacilli which are incapable of producing progressive lesions but which may cause positive reactions to the tuberculin test.

De Kock (1932) stresses the point that a tuberculin reaction signifies an infection and not necessarily a tuberculous disease and states that the absorption or harbouring of tubercle bacilli sufficient to sensitize the animal tissues does not always of necessity result in or lead to tuberculous processes visible to the naked eye.

Stenius (1933) quotes statistics from Finland collected over a period of 10 years. These show that about 0·1 per cent. of all cattle killed for food purposes were tuberculous, while on the other hand tuberculin tests showed an incidence of positive reactors of 2 per cent. to 3 per cent. Investigations carried out as a result of these findings showed that on several farms where tuberculin tests gave weakly positive reactions these cattle frequently were owned by persons affected with open tuberculosis. In some few cases the human type of *M. tuberculosis* was isolated from cattle, but usually the only indication of infection was an intradermal tuberculin reaction, although in these animals no reaction was given to the old subcutaneous test.

Hindersson (1933) while carrying out post-mortem examinations on positive reactors on many farms, encountered a number of " no lesion " cases. Suspensions of lymph glands from these animals when injected into guinea-pigs caused lesions of tuberculosis and cultures made from them revealed the human type of *M. tuberculosis.* Medical examinations of the attendants on these farms demonstrated in every case that a person attending these animals was suffering from active pulmonary tuberculosis accompanied by coughing and expectoration. Many of these reactors reacted equally well to tuberculin made from either human or bovine strains. As a result an experimental calf was fed with *M. tuberculosis* (human type) and subsequently tested. It reacted but at post-mortem examination no lesions of the disease were found. As a result of the inoculation of guinea-pigs with emulsions of the lymph glands, *Mycobacterium tuberculosis* (human type) was isolated from the lesions caused.

De Kock (1932) reviews different methods of tuberculosis eradication, viz., Bang's, Ostertag's, the 1925 Tuberculosis Order of Great Britain, and the accredited herd plan of America. Bang's method suggests an annual tuberculin test while Ostertag favours tuberculin tests every 3 months where cases have occurred. He advises that tests in South Africa should be carried out at intervals of 2 to 3 months until two consecutive negative tests are obtained. This brings up the question of a possible sensitisation of negative animals as a result of frequent injections of tuberculin.

Buxton and Glover (1939) report on this aspect of the tuberculin test. Their conclusions are that the site of injection, and for a distance of 2 to 4 inches from it, show an area of increased sensitivity for about three weeks and recommended that in the case of a re-test a distance of at least 6 inches away from the previous site of injection should be chosen. This is presupposing that the re-test takes place some few weeks after the original test.

Jackson and Diesel (1939) stated that in Natal where a number of herds were under test for the eradication of tuberculosis, in most of them all cattle had been submitted to testing on numerous occasions and the majority of them had given two consecutive negative tests. Recently at the annual testing a number of cattle that had previously given negative reactions showed swellings that simulated doubtful reactions. The increased measurement had sometimes been two and three times greater than the original skin fold figure and there had been slight pain and even a slight oedema.

Re-tesing had not assisted and on post-mortem examination of the more suspicious cases no visible lesions of tuberculosis had been found. Biological tests of glands from these had proved negative. No cases of open tuberculosis among owners or attendants had been observed. Cattle, newly introduced on to these farms, tested at the same time with the same batch of tuberculin, gave clear-cut negative reactions. A number of these doubtful reactors had been tested with avian tuberculin with definitely negative results. It is suggested that sensitisation, as a result of previous injections of tuberculin, may have taken place.

The Avian Type of *M. Tuberculosis* in Cattle.

At the present time much attention is being focussed on this type of *M. tuberculosis* in connection with doubtful reactions shown while testing animals for tuberculosis. Plum (1926) isolated the avian type of *M. tuberculosis* from the placenta of a cow that had aborted. Post-mortem examination revealed lesions in the uterus, the mesenteric lymph glands, the lung and the bronchial gland. The bovine type of the tubercle bacillus was isolated from the bronchial gland and both the bovine and avian type of *M. tuberculosis* from the lung lesions. Both Plum and van Heelsbergen consider the avian type of *M. tuberculosis* to be the commonest cause of abortion in cattle if the condition is caused by tuberculous infection.

Minett (1932) while testing cattle for the presence of Johne's disease with Johnin obtained doubtful reactions. With mammalian tuberculin very slight reactions were seen but with avian tuberculin marked positive reactions resulted. From the glands of the alimentary tract of three out of four animals *M. tuberculosis* of the avian type was isolated while in the fourth case this type of organism was obtained from the apparently normal intestinal mucous membrane. Post-mortem examination carried out on all these cases failed to reveal the presence of lesions of bovine tuberculosis or Johne's disease. Van Heelsbergen (1932) claims that avian tuberculosis occurs much more commonly in bovines than is generally diagnosed. In such cases lesions are mainly confined to the lymph glands of the digestive and other organs.

In the report of the Chief of the Bureau of Animal Industry, United States of America (1933) it is stated that evidence has been obtained that contact between cattle and tuberculous fowls could cause a sensitivity to tuberculin in cattle during the time of contact.

Gloyne (1933) found that the infection of cattle with avian *M. tuberculosis* usually takes the form of isolated lesions. He records that van Es found the avian type of organism in 11 out of 115 cattle exhibiting isolated lesions of tuberculosis. Feldman and Schlotthauer (1935) recount an experience with a herd of cattle tested regularly for 11 years with negative results. Suddenly 21 positive reactors were found. It was known that this herd was in direct contact with a flock of tuberculous fowls. Post-mortem examination was carried out on a considerable number of these reactors but no lesions of tuberculosis could be found. In the remainder of these animals small isolated lesions were found in the skin and mesenteric lymph glands at post-mortem. Material from 11 of these animals was injected into rabbits, guinea-pigs and fowls, and from three the avian type of *M. tuberculosis* was obtained.

Christiansen (1935), found that in a tuberculosis eradication campaign among cattle in the small islands round Denmark, although the avian type of

tuberculous infection was more common in young bovines (calves and heifers), it was by no means unusual for older animals to give reactions to avian tuberculin. On the average the reactions were weaker in this class of animal.

In a report issued by the Special Committee of the American Veterinary Medical Association on tuberculosis (1935) one of the conclusions arrived at is that the sensitization of cattle with the avian strain of *M. tuberculosis* may have some bearing on the " no visible lesion " problem.

Timoney (1939) gives a list of workers who have described cases of avian tuberculosis in cattle and states that in the great majority of these cases they are of the benign form. The lesions were localised and retrogressive being generally confined to associated lymph nodes of the alimentary tract, and occasionally to the uterus and subcutaneous tissues. He isolated *M. tuberculosis* of the avian type from the milk of a certain number of cows. As a result of artificially infecting a cow by means of introducing the avian type of organism into the milk cistern, this author states that in so-called low positive or doubtful reactors to bovine tuberculin, both types of tuberculin, avian and bovine, should be used. He further suggests that the avian type of *M. tuberculosis* may be found to be one of the principal offenders in the causation of many of the doubtful reactors when bovine tuberculin is injected intradermally. He comes to the conclusion that one of the characteristics of the avian type of infection is the absence of any demonstrable naked-eye lesions although infection is present in the tissues. Such animals will give a definite and pronounced reaction when injected with avian tuberculin.

In South Africa to-day many of the doubtful reactors to the intradermal test with bovine tuberculin are retested, using both bovine and avian tuberculin. As far as the writer is aware, not many cases of avian tuberculosis in bovines have so far been observed.

The Skin Lesion Cases.—Much investigational work has been carried out in order to prove or disprove the view that many so-called " no lesion " reactors are actual cases of tuberculosis of the subcutaneous tissues.

De Kock (1932) draws attention to the fact that in South Africa little or no attention has been paid to the presence or absence of skin lesions in tuberculin reacting cattle and states that in the United States of America this has formed an important and extensive study. He states that these cases often appear in herds that have been tuberculosis-free for some time and in which no contact with the disease has occurred. The lesions are found on the legs, shoulders or teats. Work carried out on organisms isolated from these lesions show that they are definitely acid-fast in character and cause reactions to tuberculin that are not always typical. Marsh, Warren and Morrow (1932) concur with these statements and assert that the subcutaneous lesions are almost confined to milking cattle and are usually to be found on the teats. Acid-fast organisms can frequently be demonstrated from these lesions and if injected into small animals can occasionally set up lesions from which acid-fast organisms can be recovered. Cultural efforts in their hands were unsuccessful and they express a doubt as to whether they are a *M. tuberculosis* strain. When tested with bovine tuberculin intradermally animals that show these lesions give varying and indefinite positive reactions. No reactions are observed when the subcutaneous or the ophthalmic tests are applied, and little, if any, response is shown when avian tuberculin is injected intradermally. From the evidence submitted the bacteria producing these subcutaneous lesions do not appear to be able to produce generalized tuberculosis.

In a report by the Chief of the Bureau of Animal Industry, United States of America for 1933, it is stated that further investigation into the skin lesion problem has been carried out and two acid-fast organisms isolated. When these were injected into test animals similar skin lesions were caused but they healed up after four months. During these four months these animals were sensitive to the tuberculin test.

Daines and Austin (1933) isolated 3 types of organisms from so-called " skin lesions " :—

(*a*) An acid-fast diphtheroid, forming dry rough colonies on agar. This most frequently caused nodules when injected into animals.

(*b*) An acid-fast diphtheroid forming moist glistening colonies on agar.

(*c*) A non-acid fast organism showing great pleomorphism.

Some cows injected with these organisms showed skin lesions some of which were typical. Intradermal tests with tuberculin on these cattle gave reactions that varied from negative to suspicious. The authors suggest that the organism (*a*) is an undescribed new species.

Continuing their work these same two authors (1934) isolated 4 groups of organisms from skin nodules of tuberculin reacting cattle, viz. :—

(1) An occasional *M. tuberculosis* strain of the bovine type.

(2) A partially or non-acid fast pleomorphic chromogenic bacillus.

(3) A pleomorphic orange coloured acid-fast chromogenic bacillus.

(4) A non-acid fast, non-chromogenic, pleomorphic bacillus.

Repeated injections into cattle of organism No. 3 caused apparently typical skin lesions. Two cows so inoculated gave definite but transitory tuberculin reactions and pronounced allergic reactions with a protein filtrate of the organism. They suggest that the No. 3 group of organisms is the cause of skin lesions and that at the same time a transitory sensitiveness to tuberculin is also set up.

Feldman (1934) made a histological study of the so-called " skin lesions " that occur in the subcutaneous tissues of cattle. This condition appears to be widely distributed throughout the United States of America. Thirty-four specimens of subcutaneous nodules were obtained from cattle that had given positive tuberculin reactions but which on post-mortem examination had revealed no lesions of tuberculosis. These specimens were irregular nodular masses varying in size from 1 to 4 cm. in diameter, and they were situated in the corium and underlying subcutaneous tissues. More than half of them showed varying degrees of calcification. From 22 out of the 34 specimens acid-fast organisms were obtained. In some of these the bacilli were numerous, in others few. In addition, in the cell debris some non-acid fast organisms were seen. Histologically the lesions bore a considerable resemblance to those of tuberculosis. The conclusion drawn was that *M. tuberculosis* was not the causal organism.

Lobel (1934) describes a nodular skin condition in buffaloes that is situated mainly in the corium. Acid-fast bacteria and granules were found in the fatty contents of the nodule. These nodules might show foci of necrosis or calcification. He was not able to cultivate the organisms. Eight of these affected buffaloes were tested with bovine tuberculin by the

ophthalmic method and three gave positive reactions. When tested by the subcutaneous method using bovine and avian tuberculin no reactions were obtained. The author concludes that this condition has nothing whatever to do with tuberculosis or the skin nodules of tuberculin reacting cattle described mainly from America. It resembles rather nodular leprosy of man and he, therefore terms the condition " lepra bubalorum ".

That skin lesions caused by *M. tuberculosis* are not confined to cases in cattle is confirmed by Aramy (1934) who states that post-mortem examination of positive reactors to the tuberculin test in pigeons revealed lesions, usually in the joints and in the skin.

Prendergast (1936) states that as a result of a survey of a very large number of tuberculin reacting cattle showing skin lesions, he failed on post-mortem examination to find internal lesions of tuberculosis in 95 per cent. of these cattle. He suggests that in these instances the intradermal reactions were not altogether typical.

Crawford (1936) makes reference to skin lesion cases and claims that the organisms found in these lesions do cause a sensitivity to tuberculin. He isolated two new acid-fast organisms of this type and in animals inoculated with them " skin lesions " were obtained. When these animals were tested with tuberculin, reactions were irregularly obtained. With avian tuberculin the reactions were stronger than with mammalian. From one of these strains a " tuberculin " was prepared and gave some positive reactions in tuberculous cattle and in cattle showing " skin lesions ". The reactions of these cattle were marked, however, when tested with American Standard tuberculin.

Robertson and Hole (1937) describe twelve " skin lesion " tuberculin reactors. On post-mortem examination nothing could be found internally and a biological examination also gave negative results. In eleven cases out of twelve acid-fast organisms were observed in the nodules. On media commonly used for the isolation of *M. tuberculosis* these organisms would not grow and animal inoculations were all negative. The conclusions come to by these authors were that these organisms caused a sensitization to tuberculin but were not necessarily the primary cause of the lesions. They admit the possibility of their being an atypical *M. tuberculosis* but incline rather to the view that they were probably of the saprophytic type of organisms causing a group sensitization to tuberculin. Brook (1937) refers to a condition in the United States of America and Canada known as " skin tuberculosis " and states that it received this name because a proportion of the affected cattle reacted to the several tuberculin tests although on post-mortem examination the animals are usually found to be free from microscopic lesions of genuine tuberculosis. He quotes Hagan (1930) who stated that Perard and Ramon (1913) were the first to describe this condition. He gives a good description of the lesions seen and mentions the site of their occurrence. From his investigations he stated that most of these cases seen were in herds that were free from genuine bovine tuberculosis and that a few cattle only were affected in a given herd at one time. Communications that he had received from field veterinary officers stated that if these lesions were removed surgically the animal lost its sensitivity to tuberculin. In personal communications from leading authorities in Sweden, Finland, Denmark, Germany and Switzerland, they all stated that the above-mentioned condition was unknown in their respective countries.

Standardization of Tuberculin.

In order to obviate doubtful reactions it has been asserted that tuberculin for use in animals should be of a definite standard. All tuberculin for human use is standardised according to the Frankfort standard, which is the old standard of Ehrlich. In the Union, tuberculin is standardised against the Frankfort standard and the strength of tuberculin recommended for field work should be at least slightly stronger but twice standard should be aimed at in order to obtain the best results.

In the second report of the Tuberculin Committee to the Medical Research Council (1928) two recommendations were made as a result of further experience in the carrying out of the tuberculin test, viz., that an increased strength of tuberculin might be employed. It was suggested that the actual dose (0·1 c.c.) should not be increased but a more potent form of tuberculin should be utilized. The second recommendation was that in order to obtain consistent results a standardized tuberculin should be used.

Buxton (1928) in an appendix to the above report gives a method of standardisation. The standard he used was his own and apparently he was not using the old Frankfort standard. Glover (1931 and 1932) as a result of having carried out a large number of double intradermal tests on cattle stressed the desirability of utilizing a standardised tuberculin.

De Kock (1932) laid down that in South Africa the test should be undertaken only under special conditions and one of these was " all tuberculin tests in any scheme adopted should be carried out with properly standardised tuberculin ". This was in an effort to eliminate the doubtful reactor to the test.

Glover (1933) being of the opinion that non-specific reactions to the double intradermal test might be caused by various ingredients contained in the media on which tuberculin was prepared, set out to manufacture tuberculins on synthetic media. These he purified by chemical means. On testing these tuberculins out on tuberculous cattle under laboratory conditions he found that they were not less potent than a " standard " tuberculin. In negative animals the reactions produced by the tuberculins made on synthetic media gave appreciably smaller reactions than did the tuberculin produced on the usual media.

Buxton and Glover (1933) confirmed these laboratory results by using the tuberculins prepared by their methods on cattle under field conditions. Dorset (1934) compared the " old " tuberculin of Koch with tuberculin prepared on a synthetic medium. A few more reactions were given to the synthetic medium tuberculin than to the " old " tuberculin, but the percentage of " no lesion " reactors on autopsy was about the same. He was also of the opinion that the stronger the tuberculin the fewer non-specific reactions were obtained.

Buxton (1934) continuing with the preparation of tuberculins on synthetic media remarked on the difference in the tuberculin sensitiveness of cattle as compared with man and other animals, and laid emphasis on the necessity for highly potent tuberculins. In the case of positive animals he found " old " tuberculin quite satisfactory but difficulties arose owing to non-specific reactions with this tuberculin in negative animals. He stated that these could be overcome by using synthetic media tuberculins. Reference

was then made to fluctuations in the sensitivity of the skin in tuberculous animals and he states that in highly allergic animals the injection of even non-specific substances may give rise to a reaction.

Bull (1936) stresses the importance of a standardized tuberculin as "old" tuberculin contains non-specific substances which will cause some degree of local reaction following intradermal injections in animals free of tuberculosis.

Buxton and Glover (1939) were asked by the Joint Tuberculosis Committee of the Medical and Agricultural Research Councils to submit a report on the methods of manufacture and properties of tuberculin in order to try to obviate difficulties in the interpretation of the double intradermal test. They admit there is no recognised "standard" tuberculin against which their synthetic tuberculin can be compared and no uniformity in the tests which are applied for the determination of the strength of such tuberculins. In this work they re-affirm their previous views on "old" tuberculin prepared on broth with regard to positive reactors and stress the likelihood of non-specific reactions when negative animals are tested.

In a leading article in the British Medical Journal of June 17th, 1939, the fact that tuberculin for the testing of cattle was not standardised is commented on and it is suggested that the veterinary experts might attempt this in the same way as tuberculin for human use is standardized.

While admitting that all the tuberculin for veterinary use should be standardised, it must not be expected that with the use of a standardised tuberculin all doubtful reactions will disappear and only clear cut negative or positive reactions will occur. This is borne out by articles contributed by medical observers who use a standardized tuberculin and there is as yet still no complete agreement on whether the Mantoux test is superior to the van Pirquet test or not.

Acid-fast Organisms not *M. Tuberculosis*.

Lydia Rabinowitsch (1897) discovered an acid-fast organism in market butter while studying the subject of tubercle bacilli in this commodity. This organism when injected into guinea-pigs caused lesions somewhat resembling those of tuberculosis. Further work, however, proved that this organism was not *M. tuberculosis*. There are a number of these acid-fast bacilli such as the smegma bacillus, *M. phlei* and Moeller's mist bacillus.

Schroeder (1926) in considering no lesion reactors came to the conclusion that other acid-fast organisms which were not *M. tuberculosis* might cause sensitization of animals to tuberculin. In 1932 Hagan and Levine worked on the pathogenicity of the saprophytic acid-fast bacilli. From the description of the lesions in guinea-pigs given these closely simulate the lesions caused by *M. tuberculosis*. Holth (1932), as quoted by Plum, states that animals affected with Brucellosis sometimes give positive reactions to the tuberculin test.

Hastings, Wisnicky, Beach and McCarter (1933) in the study of no lesion reactors, suggested that such reactors may result from some organism or organisms other than the bovine *M. tuberculosis* sensitizing cattle to tuberculin. They suggest the avian or human type of *M. tuberculosis*, bacillus of Johne's disease or the saprophytic members of the acid-fast group of organisms.

Schaefer (1935) reported on an examination of six cultures of acid-fast bacteria obtained from guinea-pigs. They resembled the avian type of *M. tuberculosis* but differed from it on serological grounds. The complement-fixation absorption method carried out on these organisms showed that they possessed components common to both bovine and avian types of *M. tuberculosis* but by absorption with bovine and avian strains these components can be eliminated leaving a third component which seems to be specific to each member of this group.

Plum (1937) states that an animal affected with the avian type or with *M. paratuberculosis* may also give a positive reaction to mammalian tuberculosis.

Timoney (1939) remarked on the greater frequency of reactions to the homologous tuberculin as compared with reactions to the heterologous product. Group reactions have been noted by various workers whom he quotes and states they are of the greatest importance to the practitioner who uses the intradermal method of tuberculin testing herds. He suggests it is highly probable that many of the so-called doubtful reactions are due to sensitization of the bovine tissues by members of the acid-fast group of bacteria other than the bovine tubercle bacillus.

Buxton and Glover (1939) carried out a series of experiments on animals by injecting saprophytic acid-fast organisms, non-acid-fast organisms and different types of *M. tuberculosis*. In the case of cattle injected with saprophytic acid-fast organisms no reactions were obtained. Some animals infected artificially with *Br. abortus* gave positive reactions when tested with tuberculin.

Personal Investigations carried out in an Attempt to Elucidate the Cause of No-lesion Reactors.

In 1934 the writer in an attempt to explain the cause of non-specific reactions experienced in the course of testing animals in the Union of South Africa by means of the double intradermal test, carried out experiments in order to find out whether saprophytic acid-fast organisms, types of *M. tuberculosis*, *Br. abortus*, Preisz-Nocard organism, and unidentified diphtheroids could cause sensitization of guinea-pigs to tuberculin prepared from human or bovine strains of *M. tuberculosis*. These results are now, for the first time, being reported on and form the main portion of this thesis.

The technique of the double intradermal test, as carried out in the Union, is identical with that carried out overseas, and whether or not the interpretation of so-called reactions differs from overseas interpretations, the fact remains that lesions of tuberculosis cannot be found in spite of very careful post-mortem examinations in numbers of cases giving reactions. In many of the cases lesions of disease are found, but they are definitely not caused by the bacillus of tuberculosis Marked reactions have been given in animals that showed extensive abscesses of the liver, in animals the subject of contagious abortion and actinomycosis and finally in animals whose post-mortem was negative and yet were close to parturition.

It was suggested that these animals might harbour acid-fast organisms, not the specific organism, and that these might be the cause of the apparent positive tuberculin reactions.

Cases have been observed in two cows where the reactions were almost identical in extent and in character, i.e., oedema, heat, tenderness, etc., and yet on post-mortem one showed characteristic lesions of tuberculosis while the other was a case of extensive liver abscessation.

I have to thank Drs. Robinson and de Kock for suggesting this work, for encouragement and for the interpretation of many of the results obtained.

Methods of Investigation involving the use of Groups of Guinea-pigs infected with Acid-fast and other Bacterial Types.

The first group of guinea-pigs was infected artificially with acid-fast organisms of the tuberculosis types while others were injected with *Brucella abortus* and strains of various diphtheroid organisms.

The second group was infected with organisms of the acid-fast type not belonging to the tuberculosis organism group, the Preisz-Nocard bacillus and a strain of *C. pyogenes*. The guinea-pigs for sensitization were infected according to the methods of Hagan and Levine (1932). One cubic centimetre of each of the various emulsions was injected either subcutaneously or by the intraperitoneal route, the object being to ascertain which of these methods would cause the better infection and be followed by sensitization to substances used in testing.

Twenty-four days after inoculation they were tested intradermally with standard tuberculin in dilutions, varying from 1:500 to 1:4000.

The following were the results:—

(i) Two groups of guinea-pigs inoculated respectively, subcutaneously and intraperitoneally with a saline emulsion of the B.C.G. strain of *M. tuberculosis*, gave marked reactions to the test.

(ii) Guinea-pigs inoculated subcutaneously in separate groups with:

(*a*) A saline emulsion of avian *M. tuberculosis;*

(*b*) a liquid paraffin emulsion of *M. phlei*;

(*c*) a liquid paraffin emulsion of avian *M. tuberculosis* showed slight reactions.

(iii) A group of guinea-pigs inoculated subcutaneously with a saline emulsion of *M. phlei* showed no reactions.

All control guinea-pigs tested were negative. A similar test was carried out a week later on the same sensitized guinea-pigs, using dilutions of from 1:50 to 1:4000 of standard tuberculin. The summarized results were:—

(i) Reactions in guinea-pigs inoculated with:

(*a*) A saline emulsion of the B.C.G. strain of *M. tuberculosis* subcutaneously;

(*b*) a liquid paraffin emulsion of *M. phlei* subcutaneously;

(*c*) a saline emulsion of the B.C.G. strain of *M. tuberculosis* intraperitoneally.

(ii) Slight reactions in guinea-pigs inoculated subcutaneously with a saline emulsion of *M. phlei.*

In addition a group of guinea-pigs, sensitized subcutaneously 60 days previously with a saline emulsion of *Br. abortus*, was tested with Onderstepoort tuberculin (Batch 5) in dilutions varying from 1:500 to 1:4000. Slight reactions to the test were given. All controls were negative.

Thirty-five days after sensitization further guinea-pigs from the same groups were tested intradermally with standard tuberculin in dilutions varying from 1:500 to 1:4000. The summarized results were as follows:—

(i) Reactions in guinea-pigs inoculated intraperitoneally with a saline emulsion of the B.C.G. strain of *M. tuberculosis*.

(ii) Slight reactions in guinea-pigs inoculated by the subcutaneous route with:

(*a*) a saline emulsion of avian *M. tuberculosis*;

(*b*) a liquid paraffin emulsion of *M. phlei*;

(*c*) a liquid paraffin emulsion of avian *M. tuberculosis*;

(*d*) a saline emulsion of *Br. abortus*.

Controls were all negative.

The following day—36 days after inoculation—the remaining sensitized guinea-pigs were tested intradermally with standard tuberculin in dilutions varying from 1:50 to 1:500. To these was added a further group of guinea-pigs inoculated intramuscularly a month previously with a saline emulsion of human *M. tuberculosis* (T 100).

The results can be summarized as follows:—

(i) Marked reactions were obtained in guinea-pigs inoculated:

(*a*) intraperitoneally with a saline emulsion of the B.C.G. strain of *M. tuberculosis;*

(*b*) intramuscularly with a saline emulsion of a human strain of *M. tuberculosis.*

(ii) Moderate reactions were shown in guinea-pigs inoculated:

(*a*) subcutaneously with a saline emulsion of the B.C.G. strain of *M. tuberculosis;*

(*b*) subcutaneously with a liquid paraffin emulsion of *M. phlei.*

(iii) Slight reactions were given in guinea-pigs inoculated:

(*a*) subcutaneously with saline emulsion of avian *M. tuberculosis*,

(*b*) subcutaneously with a liquid paraffin emulsion of avian *M. tuberculosis.*

No reactions were given with guinea-pigs inoculated:

(*a*) subcutaneously with a saline emulsion of *M. phlei*;

(*b*) intraperitoneally with a saline emulsion of *Br. abortus.*

Tests were now made on guinea-pigs inoculated 27 days previously with various diphtheroids and one group of guinea-pigs infected intramuscularly with a strain of *M. tuberculosis.* The dilutions of standard tuberculin used varied from 1:50 to 1:4000. Marked reactions were given by the guinea-pigs that were infected with the human strain of *M. tuberculosis.* In the guinea-pigs infected either subcutaneously or intraperitoneally with a saline emulsion of diphtheroids isolated from the supramammary glands of a cow

no sign of a reaction was observed. Very slight reactions were seen in one guinea-pig belonging to the group infected intraperitoneally and the group infected subcutaneously with a saline emulsion of diphtheroids obtained from a calf. Controls were negative.

To complete this group of experiments guinea-pigs already tested were subjected to further tests with dilutions of standard tuberculin varying from 1:50 to 1:400. This was fifty-six days after inoculation. Only slight reactions were given in two groups, viz.:

(*a*) inoculated with a saline emulsion of the B.C.G. strain of *M. tuberculosis* subcutaneously;

(*b*) inoculated with a similar emulsion intraperitoneally.

Summary of Results.

Twenty-four days after being infected, guinea-pigs inoculated with saline emulsions of the B.C.G. strain of *M. tuberculosis*, both by subcutaneous and intraperitoneal channels, showed marked local reactions to tuberculin injected intradermally. Slight local reactions were shown in guinea-pigs inoculated subcutaneously with saline emulsions of the avian type of *M. tuberculosis*, liquid paraffin emulsion of the same organism and liquid paraffin emulsion of *M. phlei*.

Guinea-pigs inoculated by the subcutaneous route with saline emulsions of *M. phlei*, gave no local reactions to the test with tuberculin.

The highest dilution at which the tuberculin gave marked reactions was 1:1000.

Forty-nine days after being infected local reactions to tuberculin were observed in one case of a subcutaneously inoculated guinea-pig with a saline emulsion of the B.C.G. strain of *M. tuberculosis*, the highest dilution now being only 1:100. A similar reaction, but going to a dilution of 1:250, was observed in one guinea-pig inoculated subcutaneously with a liquid paraffin emulsion of *M. phlei*. Slight, but definite tapering reactions up to 1:4000, were seen in the guinea-pigs inoculated intraperitoneally with a saline emulsion of the B.C.G. strain of *M. tuberculosis*.

Guinea-pigs inoculated subcutaneously with an emulsion of *Br. abortus* organisms, also gave definite reactions.

Thirty-five days after inoculation, guinea-pigs inoculated intraperitoneally with a saline emulsion of the B.C.G. strain gave definite reactions to dilutions of from $^1/_{500}$ to $^1/_{4000}$ of tuberculin.

Varying, very slight reactions, in the low dilutions of tuberculin, were seen in guinea-pigs:

(*a*) inoculated subcutaneously with saline emulsion of avian T.B. strain;

(*b*) inoculated subcutaneously with liquid paraffin emulsion of avian T.B. strain;

(*c*) inoculated subcutaneously with liquid paraffin emulsion of *M. phlei*.

(*d*) inoculated subcutaneously with saline emulsion of *Br. abortus* organisms.

After thirty-six days another batch of similarly infected guinea-pigs was tested with identical but more marked reactions. Guinea-pigs inoculated intramuscularly with 1 mg. of a human strain of *M. tuberculosis* gave very marked reactions. In this batch, however, the dilutions of tuberculin used varied from $^1/_{50}$ to $^1/_{500}$. An interesting result was noted in one of the control guinea-pigs. This animal gave slight but definite reactions to all dilutions of tuberculin. It was destroyed and post-mortem examination revealed almost full-time young in the uterus. This is of importance because some cows tested with tuberculin when near to calving, may give suspicious positive reactions which will not recur when a retest is carried out some time after parturition.

Results after inoculation fifty-six days previously resembled those after inoculation forty-nine days previously, as mentioned earlier in this summary.

In view of the results obtained in the first portion of this work, it was decided to carry out further experiments using acid-fast organisms, other than *M. tuberculosis*, the Preisz-Nocard bacillus and a strain of *C. pyogenes*.

The guinea-pigs were inoculated in the same way as in the previous experiment, but in every case the vehicle used for suspending the organisms for inoculation was liquid paraffin.

Forty-two guinea-pigs with controls were used, and they were grouped in the following way:—

Date of Inoculation—1.8.33.

No. of Cage.	No. of Pigs Used.	Channel of Artificial Infection.	Emulsion used and Amount.
1	3	Subcutaneous....	Liquid paraffin, *M. phlei*, 1 c.c.
	3	Intraperitoneal...	Liquid paraffin, *M. phlei*, 1 c.c.
	3	Controls.....	Controls.
2	3	Subcutaneous....	Liquid paraffin, *M. butyricum*, 1 c.c.
	3	Intraperitoneal...	Liquid paraffin, *M. butyricum*, 1 c.c.
	3	Controls.....	Controls.
3	3	Subcutaneous....	Liquid paraffin, *M. smegmatis*, 1 c.c.
	3	Intraperitoneal...	Liquid paraffin, *M. smegmatis*, 1 c.c.
	3	Controls.....	Controls.
4	3	Subcutaneous....	Liquid paraffin, Moeller s Mist Bac., 1 c.c.
	3	Intraperitoneal...	Liquid paraffin, Moeller s Mist Bac., 1 c.c.
	3	Controls.....	Controls.
5	3	Subcutaneous....	Liquid paraffin, *Corynebacterium P-Nocard* (horse), 1 c.c.
	3	Intraperitoneal...	Liquid paraffin, *Corynebacterium P-Nocard* (horse), 1 c.c.
	3	Controls.....	Controls.
6	3	Subcutaneous....	Liquid paraffin, *Corynebacterium P-Nocard* (sheep), 1 c.c.
	3	Intraperitoneal...	Liquid paraffin, *Corynebacterium P-Nocard* (sheep), 1 c.c.
	3	Controls.....	Controls.
7	3	Subcutaneous....	Liquid paraffin, *Corynebacterium pyogenes*, 1 c.c.
	3	Intraperitoneal...	Liquid paraffin, *Corynebacterium pyogenes*, 1 c.c.
	3	Controls.....	Controls.

Controls were placed in the same cages as their respective groups of inoculated guinea-pigs.

In from 20 to 21 days after inoculation, these guinea-pigs were tested with standard tuberculin in dilutions of $^1/_{50}$, $^1/_{100}$, $^1/_{250}$, $^1/_{500}$, $^1/_{1000}$, $^1/_{2000}$, $^1/_{4000}$.

Reactions were classed as in the previous experiment.

The results obtained were characterised by an almost complete absence of reactions. Summarized briefly, guinea-pigs inoculated with *C. Preisz-Nocard* and *C. pyogenes* organisms in liquid paraffin emulsions subcutaneously or intraperitoneally gave no sign of any reactions.

The remaining guinea-pigs inoculated with liquid paraffin emulsions of the non-specific acid-fast organisms either intraperitoneally or subcutaneously gave reactions varying from negative to very slight positive. Controls were negative.

Summary.

This experiment revealed the fact that these guinea-pigs, although infected with acid-fast organisms other than *M. tuberculosis*, only gave reactions in some cases when tested with standard tuberculin by the intradermal method in low dilutions.

Allergy Experiments in Guinea-pigs with Extracts of Non-pathogenic Acid-fast Bacteria.

Having had only slight reactions with tuberculin it was decided to prepare extracts of the tuberculin type from the organisms with which the guinea-pigs were infected.

The method of preparation of these extracts is given.

Preparation of Extracts from Acid-fast Organisms other than *M. tuberculosis*.

Cultures of *M. smegmatis*, *M. phlei*, Moeller's Mist bacillus and *M. butyricum* were made on serum agar slants and within 24-36 hours some had grown to such an extent, that on the water of condensation, very light pellicle material had formed. These cultures had been incubated at 37° C.

From this stage on the extracts of all these organisms were prepared in the same way as tuberculin is prepared. Within one month the growths had become so thick that they had sunk to the bottom of the flasks. The extracts varied in colour from light brown to dark brown. During the filtration of killed organisms only *M. butyricum* and *M. smegmatis* preparations had a detectable odour.

In addition to guinea-pigs inoculated with the non-pathogenic acid-fast organisms others were infected with.

(*a*) Diphtheroids from an infected udder of a cow.

(*b*) Diphtheroids from lesions in a calf.

(*c*) T 100— a human strain of *M. tuberculosis*.

These newly prepared extracts were tested on all these artificially infected guinea-pigs.

Thirty-eight days after sensitization by the intraperitoneal or the subcutaneous routes with the various non-pathogenic acid-fast organisms all these small animals were tested with the four specially prepared extracts in dilutions of 1:50 and 1:100.

The results may be briefly summarized as follows:—

Sensitizing Organism.	Results of Tests with all Extracts.
M. phlei	Good reactions.
M. butyricum	Good reactions.
M. smegmatis	Good reactions.
Moeller s Mist bacillus	Slight reactions.
C. Preisz-Nocard from horse	No reactions.
C. Preisz-Nocard from sheep	Slight temporary reactions.
C. pyogenes	Very slight reactions in two out of six guinea-pigs.
Diphtheroids from udder of cow	Very slight reactions in the case of guinea-pigs inoculated by the subcutaneous route.
Diphtheroids from calf	Similar to previous group.
M. tuberculosis (human strain)	Slight reactions in three out of four guinea-pigs.
Br. abortus	Doubtful reactions.

All controls were negative.

Summary.

From these experiments it would appear that, judging from the reactions obtained, the group of non-pathogenic acid-fast bacteria which were used have an antigenic factor in common, cross reactions being obtained with any one member against the others of the group.

No definite reactions were given with these extracts when tested against *M. tuberculosis* infected guinea-pigs. The same applies to guinea-pigs infected with the Preisz-Nocard organism, diphtheroid organisms or *Br. abortus*.

The extracts are evidently specific only for members of their own group of organisms and for no others which were tested.

It was decided to repeat the former experiment using dilutions of the extracts made from this group of non-pathogenic acid-fast organisms and similar dilutions of tuberculin.

More guinea-pigs were now taken and inoculated in the same manner with the four non-pathogenic acid-fast organisms.

Date of Inoculation, 30.10.33.

Cage.	No. of Guinea Pigs Used.	Channel of Artificial Infection.	Emulsion Used and Amount.
A.	3	Subcutaneous	*M. phlei* Liquid paraffin, 1 c.c.
	3	Intraperitoneal	*M. phlei* Liquid paraffin, 1 c.c.
	3	Controls	Controls.
B.	3	Subcutaneous	*M. butyricum* Liquid paraffin, 1 c.c.
	3	Intraperitoneal	*M. butyricum* Liquid paraffin, 1 c.c.
	3	Controls	Controls.
C.	3	Subcutaneous	*M. smegmatis* Liquid paraffin, 1 c.c.
	3	Intraperitoneal	*M. smegmatis* Liquid paraffin, 1 c.c.
	3	Controls	Controls.
D.	3	Subcutaneous	B. of Moeller (Mist) Liquid paraffin, 1 c.c.
	3	Intraperitoneal	B. of Moeller (Mist) Liquid paraffin, 1 c.c.
	3	Controls	Controls.
E.	3	Subcutaneous	*M. phlei* from lesions Liquid paraffin, 1 c.c.
	3	Intraperitoneal	*M. phlei* from lesions Liquid paraffin, 1 c.c.
	3	Controls	Controls.
F.	3	Subcutaneous	*M. butyricum* from lesions Liquid paraffin, 1 c.c.
	3	Intraperitoneal	*M. butyricum* from lesions Liquid paraffin, 1 c.c.
	3	Controls	Controls.

Controls were placed in the same cages as their respective groups of inoculated guinea-pigs.

In this group of experiments all animals were tested with the prepared extracts of the four non-pathogenic acid-fast organisms in dilutions of 1:50 and, in addition, with standard tuberculin in dilutions of 1:50 and 1:100. These tests were carried out 28 days after inoculation.

The results may be summarized as follows:—

Sensitizing Organism.	Results with all Extracts.	Results with Tuberculin.
M. phlei intraperitoneally	Definite reactions	Definite reactions.
M. phlei subcutaneously	Negative reactions	Negative reactions.
M. butyricum intraperitoneally	Negative reactions	Very slight reactions.
M. butyricum subcutaneously	Very slight reactions	Negative reactions.
M. smegmatis intraperitoneally	Definite reactions	Very slight reactions.
M. smegmatis subcutaneously	Definite reactions	Negative reactions.
Moeller's M. bacillus intraperitoneally	Slight reactions	Negative reactions.
Moeller's M. bacillus subcutaneously	Marked reactions	Negative reactions.
M. phlei from lesions-intraperitoneally	Marked reactions	Slight reactions.
M. phlei from lesions-subcutaneously	Marked reactions	Marked reactions in one case.
M. butyricum from lesions-subcutaneously.	Very slight reactions	Negative reactions.
M. butyricum from lesions-intraperitonally.	Slight reactions	Very slight reactions.

Controls were negative.

Summary.

These results confirmed those obtained in the previous experiment. Only slight reactions were given to tuberculin except in one case where the guinea-pig was infected with *M. phlei*.

It was decided to utilize the guinea-pigs that had recovered from the test of 30.10.33. These were again tested with the extracts prepared from the group of acid-fast organisms used throughout this work, and a preparation called Anaexo-Phlein prepared from *M. phlei*. This was in the place of standard tuberculin. This preparation was made on the same lines as Anaexo-tuberculin as described by Garet and Zeitoun (1933).

The method of preparation is as follows:—

A human or bovine strain of *M. tuberculosis* is grown on 5 per cent. glycerine peptone broth for four to six weeks. The broth of the culture below the scum, formed by the growth, is collected by very slow aspiration, by introducing a pipette very carefully below the pellicle growth of the bacilli. The liquid obtained should be clear and free from fragments of pellicle, which should be left intact.

This clear liquid is filtered and 0·5 per cent. formalin added. Dose for cattle 0·25 cubic centimetres for the intradermal test.

The dilution of Anaexo-phlein used was 1:50.

Sensitizing Organism.	Results with all Extracts.	Results with Anaexo-Phlein.
M. phlei intraperitoneally.....	Marked reactions.....	Marked reactions.
M. phlei subcutaneously.....	Marked reactions.....	Marked reactions.
M. butyricum intraperitoneally.....	Doubtful reactions.....	Doubtful reactions.
M. butyricum subcutaneously.....	Doubtful reactions in one guinea pig—rest negative	Doubtful reactions in one guinea pig—rest negative.
M. smegmatis intraperitoneally.....	Slight reactions.....	Slight reactions.
M. smegmatis subcutaneously.....	Slight reactions in one guinea pig—rest negative	Slight reactions in one guinea pig—rest negative.
Moeller's M. bacillus-intraperitoneally.....	Negative reactions.....	Negative reactions.
M. phlei from lesions-intraperitoneally....	Marked reactions.....	Very marked reactions.
M. phlei from lesions-subcutaneously.....	Very slight reactions....	Very slight reactions.
M. butyricum from lesions-intraperitoneally	Slight reactions.....	Slight reactions.
M. butyricum from lesions-subcutaneously.	Slight reactions.....	Very slight reactions.

Controls negative.

Summary.

Reactions were less marked in this experiment than in the previous one. More reactions were obtained with the Anaexo-phlein than with standard tuberculin.

While this thesis was being considered by the Board of Examiners an article entitled " The inter-relationship between the allergic responses of guinea-pigs sensitised with various members of the acid-fast group of organisms ", by Sahai (1941) appeared. The results given in the article were very similar to those given in the thesis. Sahai's method of infecting his guinea-pigs was by introducing measured amounts of the organism to be injected intramuscularly. It is not clear from his article whether, after he had completed his tests on the guinea-pigs, he destroyed them and ascertained whether the guinea-pigs showed lesions set up by the injection of the various acid-fast organisms used. In the work being reported on now Hagan and Levine's (1932) method was used for suspending the organisms in liquid paraffin but the injections into the guinea-pigs were either by the subcutaneous or the intraperitoneal route. It would be impossible to estimate actually what weight of organisms was injected but that there were enough organisms to infect at least a number of the guinea-pigs is shown by the post-mortem examinations made on guinea-pigs that died. In the case of numbers of guinea-pigs that were destroyed at the completion of the experiments no lesions could be found, in spite of the fact that skin reactions had been given in these cases to the various extracts that had been injected intradermally.

With these points in view it was decided to infect numbers of guinea-pigs with varying weighed amounts of *M. phlei* and *M. butyricum*. The amounts were 1 mg., 2 mg., 3 mg., and 4 mg. respectively. Instead of incorporating them in liquid paraffin as suggested by Hagan and Levine (1932) the method adopted by Glover (1941) in his work on " The Standardisation of Johnin ", was used. This consisted in weighing out the required amount of pellicle material and by thoroughly grinding it with warm sterile liquid paraffin in a mortar a satisfactory incorporation of the organism in

Post-mortems of Guinea-pigs injected with Acid-fast Organisms not M. tuberculosis *and Liquid Paraffin—Subcutaneous and Intraperitoneal.*

Date.	Cage.	Infecting Organisms.	Channel of Infection.	If Organisms obtained in Smears.	If Cultivated.	Lesions.
19/ 8/33	1	*M. phlei* and liquid paraffin.....	Intraperitoneal.	Acid-fasts numerous	Cultivated easily.	General lesions, liver, spleen, glands, large tube of fibrous tissue from point of injection to liver.
19/ 9/33	1	*M. phlei* and liquid paraffin.....	Intraperitoneal.	—	—	No lesions.
20/ 9/33	1	*M. phlei* and liquid paraffin.....	Intraperitoneal.	Acid-fasts numerous	Cultivated.....	Abscesses in internal glands.
3/ 9/33	1	*M. phlei* and liquid paraffin.....	Subcutaneous..	—	—	No lesions.
20/ 9/33	1	*M. phlei* and liquid paraffin.....	Subcutaneous..	—	—	No lesions.
20/ 9/33	1	*M. phlei* and liquid paraffin.....	Subcutaneous..	—	—	No lesions.
30/ 8/33	2	*M. rabinowitz* and liquid paraffin.....	Subcutaneous..	Acid-fasts present, numerous, also liquid paraffin	Cultivated easily. Used in subcutaneous inoculations.	Abscess, site of injection, and in liver.
9/ 9/33	2	*M. rabinowitz* and liquid paraffin.....	Subcutaneous..	—	—	No lesions.
9/ 9/33	2	*M. rabinowitz* and liquid paraffin.....	Subcutaneous..	—	—	No lesions.
16/ 9/33	2	*M. rabinowitz* and liquid paraffin.....	Intraperitoneal.	Acid-fasts present..	Cultivated easily.	Local abscess point of injection.
20/ 9/33	2	*M. rabinowitz* and liquid paraffin.....	Intraperitoneal.	Acid-fasts present..	Cultivated easily.	Local abscess point of injection.
20/ 9/33	2	*M. rabinowitz* and liquid paraffin.....	Intraperitoneal.	Unable to demonstrate in liver. Acid-fasts obtained from pus of abscesses	Not successful....	Pin-point areas throughout liver, abscesses internal glands.
6/10/33	3	*M. smegmatis* and liquid paraffin.....	Intraperitoneal.	—	—	No lesions.
31/10/33	3 Destroyed	*M. smegmatis* and liquid paraffin.....	Intraperitoneal.	Acid-fasts numerous	Cultivated easily.	Encapsulated abscesses near stomach—small white areas containing pus in liver.
31/10/33	3 Destroyed	*M. smegmatis* and liquid paraffin.....	Intraperitoneal.	—	—	No lesions.
31/10/33	3 Destroyed	*M. smegmatis* and liquid paraffin.....	Subcutaneous..	—	—	No lesions (3 guinea-pigs).
19/ 9/33	4	Moeller's mist bacillus and liquid paraffin	Intraperitoneal.	—	—	No lesions.
3/10/33	4	Moeller's mist bacillus and liquid paraffin	Intraperitoneal.	—	—	No lesions.
5/10/33	4	Moeller's mist bacillus and liquid paraffin	Intraperitoneal.	—	—	No lesions.
31/10/33	4 Destroyed	Moeller's mist bacillus and liquid paraffin	Subcutaneous..	—	—	No lesions (3 guinea-pigs).

the vehicle was obtained. The various amounts of the different organisms were injected intraperitoneally. These guinea-pigs were injected on 22nd October, 1941, and tested 26 days later with extracts prepared from *M. phlei* and *M. butyricum*. The dilutions of the two extracts used were from 1:50 to 1:400.

The results were as follows:—

Sensitizing Organism.	Amount Injected.	Test Extract.	Results.
M. phlei	1 mg.	*M. butyricum*	Fairly marked reactions.
M. phlei	2 mg.	*M. butyricum*	Marked reactions.
M. phlei	3 mg.	*M. butyricum*	Marked reactions. Very marked reactions in one case.
M. phlei	4 mg.	*M. butyricum*	Marked reactions.
M. phlei	1 mg.	*M. phlei*	Very marked reactions.
M. phlei	2 mg.	*M. phlei*	Very marked reactions.
M. phlei	3 mg.	*M. phlei*	Very marked reactions.
M. phlei	4 mg.	*M. phlei*	Very marked reactions.
M. butyricum	1 mg.	*M. phlei*	Very slight reactions.
M. butyricum	2 mg.	*M. phlei*	Slight reactions.
M. butyricum	3 mg.	*M. phlei*	Very slight reactions.
M. butyricum	4 mg.	*M. phlei*	Very slight reactions.
M. butyricum	1 mg.	*M. butyricum*	Marked reactions.
M. butyricum	2 mg.	*M. butyricum*	Marked reactions.
M. butyricum	3 mg.	*M. butyricum*	Marked reactions.
M. butyricum	4 mg.	*M. butyricum*	Marked reactions.

Controls: Negative.

From these results it appears that while 1 mg. of either *M. phlei* or *M. butyricum* will set up a certain degree of sensitization, 2 mg. and greater amounts appear to be better for this purpose. It was observed in the case of guinea-pigs sensitized by the injection of *M. butyricum*, when tested against *M. phlei* extract, that only in the 2 mg. group were slight reactions given in dilutions of $^1/_{100}$ and $^1/_{200}$, the majority reacting only to the $^1/_{50}$ dilution. On the other hand guinea-pigs sensitized by the injection of *M. phlei* when tested against *M. butyricum* extract gave many reactions to the $^1/_{200}$ dilution and a number to the $^1/_{400}$ dilution.

Sahai (1941) however, obtained only slight reactions in *M. phlei* sensitized guinea-pigs when tested against *M. butyricum* extract in $^1/_{25}$ and $^1/_{100}$ dilutions. Guinea-pigs when tested with the extract of the organism with which they were sensitized showed marked reactions. In the case of *M. phlei* sensitized guinea-pigs, even necrosis of the site of injection with *M. phlei* extract was seen. Controls were all negative. Being satisfied that these guinea-pigs were sensitized to their own special organism it was decided to test them all in dilutions varying from $^1/_{50}$ to $^1/_{400}$ with tuberculin used for testing cattle.

This was carried out 38 days after they had been inoculated with *M. phlei* and *M. butyricum*.

Date 9.12.41.—*Guinea-pigs sensitized by intraperitoneal injection of weighed amounts of* M. phlei *suspended in liquid paraffin. Tested after* 38 *days with tuberculin in varying dilutions.*

Quantities of *M. phlei* Injected.	1 mg.				2 mg.				3 mg.				4 mg.			
Dilutions of Tuberculin.....	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400
Guinea Pigs— No. 1.....	++	+	−	−												
No. 2.....	++	+	+−	−												
No. 3.....	++	+	−	−												
No. 4.....					+	+	+−	−								
No. 5.....					+	+−	+−	−								
No. 6.....					+	+−	+−	−								
No. 7.....									++	+	+−	−				
No. 8.....									++	+	+−	−				
No. 9.....									++	+	+−	−				
No. 10.....													+	+−	+−	−
No. 11.....													+	+−	+−	−
No. 12.....													+	+−	+−	−
Control— No. 13.....	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
No. 14.....	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Date 9.12.41.—*Guinea-pigs sensitized by intraperitoneal injection of weighed amounts of* M. butyricum *suspended in liquid paraffin. Tested after* 38 *days with tuberculin in varying dilutions.*

Quantities of *M. butyricum* Injected.	1 mg.				2 mg.				3 mg.				4 mg.			
Dilutions of Tuberculin.....	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400	1/50	1/100	1/200	1/400
Guinea Pigs— No. 15.....	++	+	+−	−												
No. 16.....	++	+	+−	−												
No. 17.....	+−	+−	−	−												
No. 18.....					+	+−	−	−								
No. 19.....					++	+	+−	−								
No. 20.....					+	+−	−	−								
No. 21.....									+−	−	−	−				
No. 22.....									+	+−	−	−				
No. 23.....									+−	−	−	−				
No. 24.....													++	+	+−	−
No. 25.....													+	+−	−	−
No. 26.....													+	+	+−	+−
Control— No. 27.....	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
No. 28.....	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

It will be seen that definite reactions to tuberculin were given in a number of the guinea-pigs, but in only a few cases were they marked. This is perhaps understandable seeing the lowest dilution was 1 : 50. Guinea-pigs sensitized with *M. phlei* gave more numerous and definite reactions than did those sensitized with *M. butyricum*. Controls were all negative.

It was now decided to post-mortem all guinea-pigs used in the experiment to ascertain the degree of infection set up by the injection of *M. phlei* and *M. butyricum*. Smears were made from all lesions and in a number of cases cultures were made.

The results of the post-mortems are tabulated and show that in the majority of cases abscess formation was observed at the point of inoculation and also in the inguinal glands. It was rather surprising how few showed lesions in the internal organs.

Post-mortems of Guinea-pigs Injected by Intraperitoneal Route.

No.	Organism Injected.	Amounts Injected.	Lesions.	Smears.	Cultures of Acid-fast Organisms.
1	*M. phlei*.....	1 mg.	Pin-point sized blister-like lesions in lung..... Fairly large inguinal abscess at point of injection..	Negative..... Acid-fast organisms present	Not done. Not done.
2	*M. phlei*.....	1 mg.	Similar lesions in lung to No. 1..... Inguinal gland much enlarged with centres of pus..	Negative..... Acid-fast organisms present	Not done. Cultivated.
3	*M. phlei*.....	1 mg.	Inguinal gland and point of injection show abscesses 1 pin-point whitish area liver.....	Acid-fast organisms present Negative.....	Not done. No growth.
15	*M. butyricum*.....	1 mg.	Abscess at point of injection.....	Acid-fast organisms present	Cultivated.
16	*M. butyricum*.....	1 mg.	Abscess in inguinal gland containing sang. pus.... Abscess at point of injection.....	Acid-fast organisms present Acid-fast organisms present	Cultivated. Not cultivated.
17	*M. butyricum*.....	1 mg.	Enlarged fibrous mass in inguinal region with centres of pus—pus oily	Acid-fast organisms present	Not cultivated.
4	*M. phlei*.....	2 mg.	Ruptured abscess point of injection—very little pus.	Negative.....	—
5	*M. phlei*.....	2 mg.	3 subcutaneous abscess s and abscess in inguinal gland Large abscess—liver.....	Acid-fast organisms present Acid-fast organisms very rare	Not cultivated. No growth.
6	*M. phlei*.....	2 mg.	Abscess in gland in axilla..... 3 small subcutaneous abscesses..... Large abscess in inguinal gland..... 2 pin-point whitish areas liver..... Number of irregular whitish areas throughout lungs One pin-point whitish area left kidney.....	Acid-fast organisms present Acid-fast organisms present Acid-fast organisms present Negative.....	Cultivated. Not cultivated. Not cultivated. —
18	*M. butyricum*.....	2 mg.	Large abscess inguinal gland and number of subcutaneous abscesses	Acid-fast organisms present	Not cultivated.
19	*M. butyricum*.....	2 mg.	Large abscess inguinal gland and several subcutaneous abscesses Numerous pin-point whitish areas on liver. Spleen slightly enlarged	Acid-fast organisms present Negative.....	Not cultivated. —

Post-mortems of Guinea-pigs injected by Intraperitoneal Route—(continued).

No.	Organisms Injected.	Amounts Injected.	Lesions.	Smears.	Cultures of Acid-fast Organisms.
20	*M. butyricum*.....	2 mg.	Very large abscess inguinal region also several subcutaneous abscesses and one in inguinal gland Liver dotted with pin-point whitish areas.....	Acid-fast organisms present in pus from all Negative.....	— No growth.
7	*M. phlei*.....	3 mg.	Ruptured abscess seat of injection—very little pus.	Negative.....	No growth.
8	*M. phlei*.....	3 mg.	Ruptured abscess seat of injection—very little pus.	Negative.....	No growth.
9	*M. phlei*.....	3 mg.	Ruptured abscess seat of injection—very little pus. Numerous small subcutaneous abscesses on under surface of abdominal cavity	Negative..... Acid-fast organisms present	Not cultivated. Cultivated.
21	*M. butyricum*.....	3 mg.	Ruptured abscess seat of injection. Abscess formation inguinal gland	Acid-fast organisms present	Not cultivated.
22	*M. butyricum*.....	3 mg.	Very large abscess in inguinal gland.....	Acid-fast organisms present	Cultivated.
23	*M. butyricum*.....	3 mg.	Small abscess seat of injection and one in inguinal gland	Acid-fast organisms present	Not cultivated.
10	*M. phlei*.....	4 mg.	Small subcutaneous abscess and numerous small subcutaneous abscesses between ribs on same side as injection made	Acid-fast organisms present	Cultivated.
11	*M. phlei*.....	4 mg.	Large inguinal gland abscess.....	Acid-fast organisms present	Cultivated.
12	*M. phlei*.....	4 mg.	Several small subcutaneous abscesses.....	Acid-fast organisms present	Not cultivated.
24	*M. butyricum*.....	4 mg.	Old healed subcutaneous abscess.....	—	—
25	*M. butyricum*.....	4 mg.	Small partially healed inguinal gland abscess.....	Acid-fast organisms present	Cultivated.
26	*M. butyricum*.....	4 mg.	Large subcutaneous abscess.....	Negative.....	No growth.
13 14 40 41	Controls.....	—	Negative.....	—	—

From these post-mortem results it will be seen that at the time of testing all guinea-pigs were actually infected. The doses of 3 mg. and 4 mg. of the two organisms used appear in a number of cases to set up large abscesses which rupture quickly and the guinea-pigs do not have as heavy an infection as one would like.

In order to end this part of the work it was decided to inject guinea-pigs with 2 mg. and 3 mg. of *M. phlei* and *M. butyricum* intraperitoneally and finally test these out against extracts from these organisms and also against tuberculin.

Earlier on in this paper it was stated the 2 mg. and greater amounts of the organism to be injected resulted in a higher degree of sensitization than when 1 mg. amounts were used.

Twelve guinea-pigs were injected intraperitoneally with liquid paraffin emulsions of *M. phlei* and *M. butyricum* in weights amounting to 2 mg. and 3 mg. The injection took place on 23rd January, 1942, and on 9th February, 1942, one of the guinea-pigs inoculated with 3 mg. of *M. phlei* was found dead. Post-mortem revealed a pleuritis with liquid in the chest cavity, numerous abscesses along the greater curvature of the stomach, the liver adherent to the stomach, abscess formation in the spleen, a large abscess almost enclosing bladder, and numerous abscesses in omentum, while the intestines, kidney, spleen and stomach showed adhesions between them. Five smears from abscesses in various parts of the abdominal cavity showed very frequent acid-fast organisms, many of which had a " beaded " appearance. On 3rd March, 1942, forty-three days after inoculation, these guinea-pigs were tested intradermally using extracts of *M. phlei*, *M. butyricum* and tuberculin made up in dilutions varying from 1 : 50 to 1 : 400.

The results were as follows :—

Sensitizing Organism.	Amount Injected.	Test Extract.	Results.
M. butyricum	2 mg.	*M. butyricum*	Marked reactions.
M. butyricum	3 mg.	*M. butyricum*	Marked reactions.
M. butyricum	2 mg.	*M. phlei*	Fairly marked reactions.
M. butyricum	3 mg.	*M. phlei*	Fairly marked reactions.
M. butyricum	2 mg.	Tuberculin	Slight reactions in dilutions of 1 : 200.
M. butyricum	3 mg.	Tuberculin	Slight reactions.
		Controls : Negative.	
M. phlei	2 mg.	*M. phlei*	Marked reactions.
M. phlei	3 mg.	*M. phlei*	Very marked reactions.
M. phlei	2 mg.	*M. butyricum*	Fairly marked reactions.
M. phlei	3 mg.	*M. butyricum*	Fairly marked reactions.
M. phlei	2 mg.	Tuberculin	Slight reactions in dilutions of 1 : 100.
M. phlei	3 mg.	Tuberculin	Slight reactions in dilutions of 1 : 200.
		Controls : Negative.	—

Each group reacted very strongly to its homologous extract and although reactions were obtained with the tuberculin they were not so marked as the reactions to the extracts of *M. phlei* and *M. butyricum*.

Ten days later all these guinea-pigs were tested alone with tuberculin in dilutions varying from 1:10 to 1:80; in nine out of eleven guinea-pigs good reactions were given. All were submitted to post-mortem examination and the results are appended.

Date 13.3.42.—*Guinea-pigs sensitized by intraperitoneal injection of weighed amounts of* M. phlei *suspended in liquid paraffin. Tested after* 53 *days. Tested with tuberculin in varying dilutions.*

Extract.	Tuberculin.							
Amounts of *M. phlei* Injected.	2 mg.				3 mg.			
Dilutions	1/10	1/20	1/40	1/80	1/10	1/20	1/40	1/80
Guinea-pig 35	++	++	++	+				
Guinea-pig 36	++	++	++	+				
Guinea-pig 37	++	++	+	+−				
Guinea-pig 38					++	++	++	++
Guinea-pig 39					+	+	+−	−
Guinea-pig 40		D		E		A		D
Controls	−	−	−	−	−	−	−	−

Date 13.3.42.—*Guinea-pigs sensitized by intraperitoneal injection of weighed amounts of* M. butyricum *suspended in liquid paraffin. Tested after* 53 *days. Tested with tuberculin in varying dilutions.*

Extract.	Tuberculin.							
Amounts of *M. butyricum* Injected.	2 mg.				3 mg.			
Dilutions	1/10	1/20	1/40	1/80	1/10	1/20	1/40	1/80
Guinea-pig 29	+++	++	++	+				
Guinea-pig 30	+	+−	−	−				
Guinea-pig 31	++	++	++	+				
Guinea-pig 32					++	++	+	+
Guinea-pig 33					++	+	+	+
Guinea-pig 34					++	++	+	+
Controls	−	−	−	−	−	−	−	−

Post-mortems of Guinea-pigs injected by Intraperitoneal Route.

No.	Organisms Injected.	Amounts Injected.	Lesions.	Smears.	Cultures of Acid-fast Organisms.
28	*M. phlei*.....	2 mg.	Large abscess at site of injection and abscesses in inguinal gland	Acid-fast organisms present in smears from both lesions	Not done.
29	*M. phlei*.....	2 mg.	Large abscess in inguinal gland..... Small whitish pin-point area in spleen pulp.....	Acid-fast organisms present No organisms seen.....	Cultivated. —
30	*M. phlei*.....	2 mg.	Several large abscesses in inguinal gland..... Both lungs show large number of small white tough areas that are easily enucleated	Acid-fast organisms present No organisms seen.....	Not done. Not done.
31	*M. phlei*.....	3 mg.	Abscess, large at point of injection..... Small abscess inguinal gland.....	Acid-fast organisms present No organisms seen.....	Not done. Not done.
32	*M. phlei*.....	3 mg.	Two large abscesses at site of injection and large abscess in inguinal gland	Acid-fast organisms present	No growth.
33	*M. phlei*.....	3 mg.	Died, 9/2/42. No post-mortem.....	—	—
34	*M. butyricum*.....	2 mg.	Abscess in inguinal gland.....	Acid-fast organisms present	Cultivated.
35	*M. butyricum*.....	2 mg.	Large abscess site of injection..... Small abscesses over outer surface of ribs..... Whole carcase emaciated.....	Acid-fast organisms present	Not done.
36	*M. butyricum*.....	2 mg.	Large abscess site of injection.....	Acid-fast organisms present	Cultivated.
37	*M. butyricum*.....	3 mg.	Large abscess site of injection..... Liver shows numerous small whitish areas.....	Acid-fast organisms seen... No organisms seen.....	Not done. —
38	*M. butyricum*.....	3 mg.	Abscess site of injection..... Liver shows numerous small whitish areas..... Abscesses in omentum.....	Acid-fast organisms seen... No organisms seen..... Acid-fast organisms present	Not done.
39	*M. butyricum*.....	3 mg.	Abscess site of injection.....	Acid-fast organisms seen...	Cultivated.
40 41	Controls.....	—	Negative.....	—	—

Experiments on the Sensitization of Calves to Acid-Fast Organisms other than *M. tuberculosis*.

Following the results obtained in the guinea-pigs it was decided to take a number of calves varying in age from six months to two years and see if by sensitizing them to *M. phlei* and *M. butyricum* they would give any reactions when tested intradermally with tuberculin. As a first step they were submitted to the double intradermal tuberculin test in order to be sure that they were not subjects of tuberculosis. The results are given below.

Calf.	Date.			Results and Remarks.
	20/10/41.	22/10/41.	23/10/41.	
No. 648	5 mm.	5 mm.	8 mm.	Negative. Circumscribed.
No. 645	5 mm.	5 mm.	7 mm.	Negative. Circumscribed.
No. 656	5 mm.	5 mm.	6 mm.	Negative. Circumscribed.
No. 665	5 mm.	5 mm.	7 mm.	Negative. Circumscribed.
No. 668	4 mm.	4 mm.	6 mm.	Negative. Circumscribed.
No. 669	4 mm.	4 mm.	6 mm.	Negative. Circumscribed.
No. 671	4 mm.	4 mm.	6 mm.	Negative. Circumscribed.
No. 670	4 mm.	4 mm.	5 mm.	Negative. Circumscribed.
No. 672	4 mm.	4 mm.	5 mm.	Negative. Circumscribed.
No. 676	4 mm.	4 mm.	4 mm.	Negative. Circumscribed.
No. 678	4 mm.	5 mm.	5 mm.	Negative. Circumscribed.
No. 674	4 mm.	5 mm.	5 mm.	Negative. Circumscribed.
No. 675	4 mm.	4 mm.	6 mm.	Negative. Circumscribed.
No. 680	4 mm.	5 mm.	4 mm.	Negative. Circumscribed.
No. 679	5 mm.	6 mm.	5 mm.	Negative. Circumscribed.

These were then divided into six groups and inoculated subcutaneously—group 1 received 10 mg. of *M. phlei*, group 2 received 10 mg. of *M. butyricum*, group 3 received 30 mg. of *M. phlei*, group 4 received 30 mg. of *M. butyricum*, group 5 received 90 mg. of *M. phlei* and group 6 received 90 mg of *M. butyricum*. As in the case of the guinea-pigs the organisms were suspended in liquid paraffin according to the method of Glover (1941). The varying amounts were then injected under the skin behind the shoulder. For a few days after inoculation most of the animals were slightly lame and the injection site tender, but they soon recovered.

On 17.12.41 and 19.12.41, forty-seven days later each calf was tested by the double intradermal test with extracts prepared from *M. phlei* and *M. butyricum*.

On 5.1.42, the double intradermal test with tuberculin was carried out to see whether these animals were sensitive to tuberculin.

Calf.	Organisms Injected.	Amounts Injected.	*Butyricum* Extract. Dates.				*Phlei* Extract. Dates.				Tuberculin.			Tuberculin.				Remarks.
			17/12/41.	18/12/41.	19/12/41.	20/12/41.	17/12/41.	18/12/41.	19/12/41.	20/12/41.	20/10/41.	22/10/41.	23/10/41.	5/1/42.	6/1/42.	7/1/42.	8/1/42.	Reaction to Tuberculin.
			mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	
668	*M. butyricum*	10 mg.	5	14	11	28	5	11	9	24	4	4	6	5	9	7	9	Circumscribed.
680	*M. butyricum*	10 mg.	5	13	12	23	4	17	11	15	4	5	4	4	6	5	9	Circumscribed.
669	*M. butyricum*	30 mg.	5	21	35	Oe. 51	4	9	11	22	4	4	6	4	10	9	10	Circumscribed.
676	*M. butyricum*	30 mg.	5	16	20	27	4	8	8	10	4	4	4	4	7	6	9	Circumscribed.
670	*M. butyricum*	90 mg.	4	15	17	37	4	11	13	26	4	4	5	5	7	7	8	Circumscribed.
665	*M. butyricum*	90 mg.	7	15	18	37	6	10	12	22	5	5	7	5	8	9	9	Circumscribed.
679	*M. phlei*	10 mg.	4	13	10	26	4	16	17	20	5	6	5	4	6	5	7	Circumscribed.
678	*M. phlei*	10 mg.	4	8	8	14	3	10	10	21	4	5	5	5	5	5	9	Circumscribed.
675	*M. phlei*	30 mg.	5	10	10	29	3	14	18	16	4	4	6	4	6	6	7	Circumscribed.
674	*M. phlei*	30 mg.	5	10	8	15	4	22	22	Oe. 26	4	5	5	4	6	5	6	Circumscribed.
672	*M. phlei*	90 mg.	4	13	9	18	4	13	12	22	4	4	5	4	5	5	5	Circumscribed.
671	*M. phlei*	90 mg.	5	11	9	20	4	15	17	Oe. 26	4	4	6	4	6	5	6	Circumscribed.
648	Control	—	7	11	10	11	6	9	8	7	5	5	8	6	7	6	7	Circumscribed.
656	Control	—	9	12	11	13	7	10	8	9	5	5	6	6	7	6	7	Circumscribed.

Oe = Oedema.

Results.—It appears that animals can be sensitized by as small a weight of infecting organisms as 10 mg. given subcutaneously. Those animals, however, that received 30 mg. or 90 mg. gave more pronounced skin reactions. At the time of testing, the site of injection behind the shoulder in all cases felt like an irregular indurated mass of fibrous tissue under the skin. This projected only very slightly above the skin level. At the time of the reactions to the two injected extracts the prescapular glands on both sides of the body were enlarged but movable. They were also slightly painful. The animals injected with *M. butyricum* gave more marked skin reactions when tested with butyricum extract than did the animals injected with *M. phlei* when tested with phlei extract. The increase in skin measurements were in some cases very marked, e.g., from 5 mm. to 51 mm., 4 mm. to 37 mm., 4 mm. to 26 mm. For the most part they simulated definite positive tuberculin reactions. The sites of injection showed oedema, were hot and tender to the touch and the prescapular lymph glands were enlarged. In no case was a systemic reaction observed in any of the animals. These reactions would entitle one to claim that these animals had been sensitized.

Sixteen days later a second intradermal tuberculin test was carried out on these sensitized animals, the actual injections being made on the opposite side of the neck to that used for the butyricum and phlei extracts. The skin enlargements, as a result of the tuberculin injected, were, by no means, so marked as in the case of *M. phlei* and *M. butyricum* extracts injected. In some few cases the measurements of the skin were more than double and in the remainder the increase varied from 1 mm. to 4 mm. In all cases, however, the local reactions were strictly circumscribed. Oedema was not present, even in those cases where the skin reaction was double the size of the original skin measurement. When one compares the results of this tuberculin test carried out more than two months previously one would be entitled to assert that reactions had been given to the tuberculin as a result of having sensitized the animals with either *M. phlei* or *M. butyricum*. The question to be answered now is whether those reactions would be regarded as suspicious or positive if they occurred as a result of a double intradermal tuberculin test in an animal that had not been deliberately sensitized with any non-pathogenic acid-fast organisms. If one relied solely on the character of the swelling, the opinion would probably be expressed that the reactions were only slightly suspicious, tending towards negative. Various workers in different parts of the world place much stress on the size of the swellings set up by the injections of tuberculin. Buxton and MacNalty (1928) give figures for average skin measurements increase in 39 non-tuberculous animals as 3 mm. and for 39 tuberculous animals as 12·74 mm., but they finally state that it was impossible to lay down any definite increase in skin measurement which could be taken as absolutely diagnostic of tuberculosis.

Christiansen (1933) maintained that an increase in skin measurement of 2·5 or over in a herd believed to be free of tuberculosis was indicative of a positive reactor. In other herds an increase of 3·5 or over between the two skin measurements was an indication of a positive reactor while an increase of 1·5 mm. to 3·5 mm. suggested a suspicious case.

Zeller (1936) decided that an increase of 3 mm. in the skin measurement indicated a positive reactor. There are thus three groups of workers who interpret reactions differently. Group one relies on the clinical appearance of the reaction, group two gives an opinion on the combination of the character of the swelling together with its measurements while group three appears to rely solely on the increase in the size of the skin measurements.

To sum up fairly on these lines one would say that the tuberculin reactions given by the calves sensitized with *M. phlei* and *M. butyricum* would be negative, if judged solely on the clinical appearance; if judged by clinical appearance plus measurements they would be regarded as suspicious, while finally if measurement alone was the criterion they would be classed as positive.

As most experienced workers in tuberculin testing interpret results on the character of the swelling at the site of injection together with the increase in its measurement, six of the calves in this experiment would be classed as suspicious tuberculosis cases. These results lend support to the views of such workers as Schroeder (1926), Hastings, Wisnicky, Beach and McCarter (1933) and Timoney (1939), that cattle sensitized with non-pathogenic acid-fast organisms may give reactions simulating positive tuberculin reaction when tested with this extract.

Eleven months after inoculation the remaining calves from the previous experiment were once more tested with extracts prepared from *M. butyricum*, *M. phlei* and a bovine strain of *M. tuberculosis*. Thirteen and a half months after inoculation these same animals were tested with avian tuberculin. All the results are concentrated in one table for the sake of comparison and brevity. The sizes of the skin swellings as a result of the original injections are also given (see following page).

The results of the avian tuberculin tests were rather disappointing as it was anticipated that the reactions given would be greater than those given to the bovine tuberculin test, especially in view of the article on " The Tuberculin Test " issued by the British Ministry of Agriculture and Fisheries in April, 1942.

In order to be satisfied that the calves used were infected it was decided to operate on them and remove the swellings. This was done on 10th December, 1942. The results were as follows:—

Calf 665.—Large encapsulated abscess 70 mm. in width, soft at one point on inner side. On sectioning it further, a number of channels were found running to smaller abscesses. The pus on being stained showed very numerous groups of acid-fast bacilli. Cultures became overgrown—no colonies of acid-fast organisms were obtained.

Calf 676.—This mass was smaller, being 32 mm. in width. This was made up of a number of encapsulated abscesses which had the appearance of tuberculous abscesses usually found in affected glands. Smears made from the pus showed very numerous acid-fast bacilli scattered through it. These were not in clumps as in smears from Calf 665. Cultures were over-grown.

Calf 670.—This mass measured 57 mm. in width. On section it was one mass of abscesses, varying in size from a pin's head to that of a pea. The pus showed very numerous acid-fast bacilli. Several colonies of acid-fast bacilli were grown on serum agar from the pus.

Calf 669.—This mass measured 51 mm. in width and the lesions resembled those seen from Calf 670. Acid-fast organisms were very numerous in clumps in pus smears. No attempts at cultivation were made. Section of the lesions showed acid-fast organisms and changes indicative of tuberculosis were noted.

Calf.	Organisms Injected.	Amounts Injected.	*Butyricum* Extract. Dates.				*Phlei* Extract. Dates.				Bovine Tuberculin. Dates.				Avian Tuberculin. Dates.			Size of Skin Swelling.
			22/9/42.	23/9/42.	24/9/42.	25/9/42.	22/9/42.	23/9/42.	24/9/42.	25/9/42.	22/9/42.	23/9/42.	24/9/42.	25/9/42.	2/12/42.	4/12/42.	5/12/42.	5/12/42.
			mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	
668	*M. butyricum*.....	10 mg.	6	8	7	12	6	11	15	19	6	7	6	6	5	6	8	Negligible.
680	*M. butyricum*.....	10 mg.	4	5	4	8	4	7	8	10	4	4	4	6	5	5	6	Negligible.
669	*M. butyricum*.....	30 mg.	7	13	10	14	7	13	10	17	7	10	7	8	5	5	6	61 mm.
676	*M. butyricum*.....	30 mg.	5	11	9	18	5	8	12	16	5	5	5	8	5	5	7	42 mm.
670	*M. butyricum*.....	90 mg.	7	10	12	22	7	13	12	27	7	7	6	10	5	6	9	67 mm.
665	*M. butyricum*.....	90 mg.	10	12	11	14	10	10	12	15	9	9	9	9	8	8	11	90 mm.
674	*M. phlei*.....	30 mg.	6	8	7	9	6	26	20	26	6	7	7	7	5	6	6	36 mm.
672	*M. phlei*.....	90 mg.	5	7	7	16	5	16	20	31	5	6	5	6	5	6	7	Negligible.
648	Control.....	—	7	10	11	10	7	9	8	7	6	7	6	6	6	7	7	Negligible.
656	Control.....	—	9	12	11	12	7	9	8	7	7	8	7	7	7	8	8	Negligible.

Calf 674 showed a systemic reaction 24 hours after injection with *Phlei* extract. It had a temperature of 105°, refused food and would not stand. An uneventful recovery was made.
Dates of injections of test extracts 22/9/42 and 24/9/42.
Dates of injection of avian tuberculin 2/12/42 and 4/12/42.

Three months have elapsed from the time of operation and except for slight scar formation the wounds have healed well. These calves will be tested with extracts prepared from *M. butyricum*, *M. phlei*, bovine tuberculin and avian tuberculin.

On 19th April, 1943.—Six of the remaining animals with a control were tested with the four extracts mentioned above. The object was to see whether the surgical removal of the lesions would result in a loss of sensitivity to any of the extracts used. This experiment was suggested in view of the work of Brook (1937). He stated that in communications he had received from field veterinary officers it was stated that if the " skin lesions " were removed surgically the animal lost its sensitivity to tuberculin.

The lesions produced artificially in these calves were of a more serious nature than ordinary " skin lesions ", as they involved the formation of large fibrous masses but in spite of this it was worth testing out after their surgical removal.

The results are given on the next page.

The results of this test do not differ greatly from those obtained on 22.9.42. It would appear that the removal of the lesions has not affected the sensitivity of the animals towards extracts prepared from *M. butyricum* and *M. phlei*. The reactions towards avian tuberculin are slightly more marked than those shown at the previous test and some might be classed as suspicious. The reactions given to the bovine tuberculin injections could still, in four cases, be classed as suspicious.

Conclusions.

1. In guinea-pigs a sufficient degree of infection with the organism of tuberculosis can be set up by artificial means so that reactions with tuberculin are given within from 24 to 30 days. This means that the period of 6-8 weeks usually elapsing before subjects, injected with suspected tubercular material are killed for diagnosis can be shortened by testing the subject and a control, with tuberculin in from 24 to 30 days, thus expediting results of tests.

2. Animals infected with *Br. abortus* may give strongly suspicious reactions when tested with tuberculin.

3. Reactions strongly suspicious of positive tuberculin reactions have been observed in cattle that were pregnant and close to the time of parturition. A similar case was observed in a control guinea-pig.

4. Guinea-pigs infected with strains of non-pathogenic acid-fast organisms gave reactions in some cases when tested with tuberculin in a dilution of 1 to 100. More marked reactions to tuberculin were obtained when lower dilutions were used, viz., 1 to 10 to 1 to 40 dilutions.

5. Extracts prepared from non-pathogenic acid-fast organisms, in the same way as tuberculin is prepared, appear to contain some antigenic factor common to all members of this group that gives rise to reactions when inoculated into small animals artificially infected with separate members of this group.

6. Extracts prepared on the lines of L'anaexo-Tuberculin may contain products that are specific for each member of the acid-fast group of organisms.

Calf.	Organisms Injected.	Amounts Injected.	*Bovine Tuberculin.* Dates.						*Avian Tuberculin.* Dates.						*Butyricum* Extract. Dates.						*Phlei* Extract. Dates.					
			19/4/43.	20/4/43.	21/4/43.	22/4/43.	23/4/43.	24/4/43.	19/4/43.	20/4/43.	21/4/43.	22/4/43.	23/4/43.	24/4/43.	19/4/43.	20/4/43.	21/4/43.	22/4/43.	23/4/43.	24/4/43.	19/4/43.	20/4/43.	21/4/43.	22/4/43.	23/4/43.	24/4/43.
		mg.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
665	*M. butyricum*	90	8	10	9	12	9	8	8	9	8	11	9	9	8	11	13	22	18	15	10	11	12	20	16	17
676	*M. butyricum*	30	4	6	5	8	5	5	5	6	5	8	5	5	5	7	8	14	10	10	6	9	10	19	13	12
670	*M. butyricum*	90	6	7	7	11	8	7	7	7	7	11	9	8	6	8	9	18	12	12	8	10	11	23	18	18
669	*M. butyricum*	30	5	7	6	9	6	6	6	8	7	10	7	7	5	8	7	16	13	12	7	9	9	15	13	14
672	*M. phlei*.....	90	6	7	7	8	6	6	6	6	6	9	6	6	7	9	8	12	10	10	9	14	13	23	17	12
680	*M. butyricum*	10	4	5	5	7	5	5	4	5	5	8	5	5	5	5	5	11	8	7	6	6	7	15	10	10
—	Control.....	—	5	5	5	7	5	5	5	5	5	7	5	5	5	7	6	11	8	8	6	7	6	11	7	7

Dates of injections of test extracts 19/4/43 and 21/4/43.

7. Cattle up to 2½ years of age can be sensitized by the subcutaneous inoculation of 10 mg. of strains of *M. butyricum* and *M. phlei* suspended in liquid paraffin. Larger amounts of the organisms do not appear to give rise to a greater degree of sensitivity.

8. Cattle sensitized by subcutaneous inoculation of strains of *M. butyricum* and *M. phlei* gave definite suspicious reactions when tested with tuberculin by the double intradermal method.

9. These same animals when tested with avian tuberculin showed no more marked suspicious reactions than when tested with standard tuberculin.

10. Thirteen and a half months after subcutaneous inoculation with strains of *M. butyricum* the non-pathogenic organism *M. butyricum* could be cultivated from pus obtained from the resultant lesion.

11. Sections cut from the lesions set up by these two organisms showed changes indistinguishable from those set up by tuberculosis.

12. Four months after the surgical removal of the lesions set up by the inoculation of the non-pathogenic acid-fast organisms the animals still gave definite positive reactions when tested against the corresponding extracts.

13. It is probable that some non-specific reactions shown by animals, when tested with tuberculin, may be due to these animals being sensitized naturally by a strain or strains of some non-pathogenic acid-fast organism.

14. This may also be an explanation for some of the "no lesion" reactors to the double intradermal tuberculin test.

LITERATURE CITED.

ARANY, G. (1934). Diagnosis of Tuberculosis in pigeons Abst. from *Abst. in Bull. Inst. Pasteur* No. 33, p. 352. Summary in *Vet. Bulletin*, September, 1935. Vol. 5, No. 9.

BANG, AND SALOMONSEN (1891). *Berl. tierarztl. Woch.* April, 6. 9. quoted by Calmette. A. 1923. Tubercle Bacillus infection and Tuberculosis in man and animals.

BUXTON, J. B. (1925). Tuberculin Tests in cattle with special reference to the Intradermal Test. *Med. Research Council special report series*, No. 94. London H.M. Stationery Office.

BUXTON, J. (1928). A note on the potency of Tuberculin. Appendix II. *Special Report Series*, No. 122, *to Medical Research Council.* London H.M. Stationery Office.

BUXTON, J. (1934). Some aspects of the role of Tuberculin in the control of Tuberculosis. *Proc. R. Soc. Med.*, Vol. 28, p. 257.

BUXTON, J. B., AND MACNALTY, A. S. (1928). The Intradermal Tuberculin test in cattle. *Med. Research Council special report series*, No. 122. London H.M. Stationery Office.

BUXTON, J. B., AND GLOVER, R. E. (1933). Comparative Field trials of a synthetic and a standard Tuberculin. *3rd report. Direct. Instit. Animal Path.*, University of Cambridge, 1932-1933.

BUXTON, J. B., AND GLOVER, R. E. (1939). Tuberculin Tests in Cattle. *Agric. Research Council A.R.C. Report Series*, No. 4. London H.M. Stationery Office.

BULL, L. B. (1936). The standardisation of Tuberculin for use in animal practice. *Aust. Vet. Jnl.*, Vol. 12, p. 168.

BROOK, G. B. (1937). Subcutaneous and cutaneous lesions in cattle which sometimes cause hypersensitiveness to Tuberculin (so called skin tuberculosis). *Vet. Jnl.*, Vol. 93, No. 4, p. 135.

CALMETTE (1907). *Compt. rend. Acad. des Sci.*, Vol. 144, p. 1324, quoted by Calmette (1923) Tubercle Bacillus infection and Tuberculosis in man and animals.

CHALMERS, J. (1927). Discusion on P. R. Viljoen's paper, Tuberculosis in S. Africa. *Jl. S.A.V.M.A.*, Vol. 1, p. 20.

CHRISTIANSEN, M. J. (1933). Denmark: Notice of 28th July, 1933, on control of Tuberculosis in cattle and swine. *Skand. Vet. Tidskr.* 23, 456-464. Summary in *Vet. Bulletin.* August, 1935. Vol. 5, No. 8.

CHRISTIANSEN, M. J. (1935). Control of Bovine Tuberculosis on the smaller islands and among suppliers to co-operative Dairies in Denmark. Avian type of infection in cattle in these areas. *Maanedsskr. Dyrlaeg*, Vol. 47, p. 321-36. Summary in *Vet Bulletin*, May, 1936. Vol. 6, No. 5.

CRAWFORD, A. B. (1936). Tuberculin reactions in cattle showing no visible Tuberculous lesions on post-mortem. *Jnl. Amer. Vet. Med. Assocn.*, Vol. 89, p. 562.

DAINES, L. L., AND AUSTIN, H. (1933). A study of so-called skin lesions of Tuberculin, reacting cattle. *Amer. Rev. Tuberc.*, Vol. 27, No. 10, p. 600.

DAINES, L. L., AND AUSTIN, H. (1934). Acid fast and non-acid fast micro-organisms in skin nodules of Tuberculin reacting cattle. *Amer. Rev. Tuberc.*, Vol. 30, p. 209.

DE KOCK, G. v. D. W. (1932). The Bovine Tuberculosis Problem in South Africa. *Jnl. S.A.V.M.A.*, July, Vol. 3, Nos. 2 and 3, pp. 53-65 and 97-105.

DORSET, M. (1934). A comparison of Koch's old Tuberculin with a new synthetic medium Tuberculin. *Jnl. Amer. Vet. Med. Assocn.*, Vol. 84, p. 439.

DOUGLAS, A. (1937). The control of Tuberculosis by means of the Tuberculin Test. *Vet. Record*, Vol. 49, No. 36, p. 1129.

FAVERO, AND FINZI (1938). Quoted by Hutyra, Marek and Manninger. Special Pathology Therapeutics of the Diseases of Domestic Animals, Vol. 1.

FELDMAN, W. H. (1934). Subcutaneous Tuberculoid lesions of cattle. A morphological study. *Arch. Path. Lab. Med.*, Vol. 17, p. 533.

FELDMAN, W. H., AND SCHLOTTHAUER, C. F. (1935). The isolation of the organism of Avian Tuberculosis from cattle. *Jnl. Amer. Vet. Med. Assocn.*, Vol 86, No. 43, p. 635.

GARET, AND ZEITON (1933). L'anaexotuberculine. *Rec. de Med. Vet. de l'Ecole Alfort.* 109. 9. 513-518. Summary in *Vet. Rec.*, 1933, Vol. 13, No. 48.

GLOVER, H. R. (1931). The Double Intradermal Tuberculin Test. *Vet. Record*, Vol. 11, p. 1158.

GLOVER, H. R. (1932). Tuberculin testing. *Vet. Record*, Vol. 12, p. 390.

GLOVER, R. E. (1933). A study on Tuberculin with special reference to the Double Intradermal Test. *3rd report Direct. Instit. Animal Path.* University of Cambridge, 1932-1933.

GLOVER, R. E. (1941). The standardisation of Johnin. *Vet. Journal*, Vol. 97, No. 1, p. 3.

GLOYNE, S. R. (1933). Avian tuberculosis with special reference to infection in man. *Bulletin of Hygiene*, No. 8.

GOODALL, A. (1927). Discussion on P. R. Viljoen's paper. Tuberculosis in S. Africa. *Jnl. of S.A.V.M.A.*, Vol 1, p. 20.

GÖTZE, R., AND MULLER, J. (1936). Zur Methodik und Technik der Tuberkulinisierung beim Rinde. *Berl. tierarztl. Wochschr*, Vol. 52, pp. 151-155.

GREEN, W. J. (1933). Notes on the Double Intradermal Tuberculin Test carried out in Durban and neighbourhood. *Jnl. S.A.V.M.A.*, Vol. 4, No. 1, pp. 27-31.

HAGAN, W. A. (1930). The skin lesion problem in the Tuberculosis eradication programme. *Agric. Bulletin*, No. 242, pp. 106-114. Proceedings of the Eleventh Annual Eastern States Conference on Tuberculosis and other animal diseases. Dept. of Agriculture and Markets. New York State.

HAGAN, W. A., AND LEVINE, P. (1932). The pathogenicity of the saprophytic acid fast bacilli. *Jnl. Amer Vet. Med. Assocn.*, Vol. 81, No. 4.

HANCOCK, R. C. G. (1939). The Elimination of some variable factors from the Intradermal Tuberculin Test. *Vet. Record*, Vol. 51, No. 1.

HASTINGS, E. G., WISNICKY, B. A., BEACH, B. A., AND McCARTER, J. (1933). A detailed study of 'no lesion' Tuberculin reacting cattle. *Jnl. Amer. Vet. Med. Assocn.*, Vol. 82, p. 565.

HENRY, M. (1936). The application of the Tuberculin Test. *Aust. Vet. J.*, Vol. 12, p. 175.

HINDERSSON, R. (1933). The occurrence of M. tuberculosis infection (Human type) in cattle. *Suomen Elain-lehti.* 39. 154-162.. Summary in *Vet. Bulletin*, Vol. 4, Nov., 1934, No. 11.

HOLTH(1932). *Norsk. Vet. Tidsskr.*, Vol. 44, p. 196.

JACKSON, S., AND DIESEL, A. M. (1939). Personal communication to author.

JANUSCHKE (1938). Quoted by Hutyra, Marek and Manninger. Special Pathology Therapeutics of the Diseases of Domestic Animals, 1938, Vol. 1, p. 637.

JORGENSON, M. (1937). Factors influencing the accuracy of the Intradermal Tuberculin Test. *Maanedsskr. Dyrlaeg.* 48, pp. 285-298. Summar. in the *Vet. Bulletin*, Vol. 7, No. 6, p. 285.

KOCH (1882). *Berl. klin. Wch.*, 19, 221, quoted by Calmette. A. 1923. Tubercle Bacillus infection and Tuberculosis in man and animals.

KOCH (1890). *Deut. med. Wch.*, 14, 1029, quoted by Calmette. A. 1923. Tubercle Bacillus infection and Tuberculosis in man and animals.

KOCH (1891). *Deut. med. Wch.*, 17, 101, quoted by Calmette. A. 1923 Tubercle Bacillus infection and Tuberculosis in man and animals.

KOCH (1897). *Deut. med. Wch.*, 23, 209, quoted by Calmette. A. 1923. Tubercle Bacillus infection and Tuberculosis in man and animals.

LAENKHOLM, W. (1934). Tuberculin Tests on Cattle (Denmark). *Medlemsbl. danske Dyrlaegeforen*, 17. 587-603. Summary in *Vet. Bulletin*, September, 1935, Vol. 5, No. 9.

LIGNIERES (1908). *Centralbl. f. Bakt.*, 46, 373, quoted by Calmette. A. 1923. Tubercle Bacillus infection and Tuberculosis in man and animals.

LLEWELYN JONES, H. (1939). Measuring the Tuberculin Reaction. *Vet. Record*, Vol. 51, No. 6, p. 190.

LOBEL, L. W. M. (1934). Lepra bubalorum, summary in *Ned.-Ind. Bl. Diergeneesk.*, Vol. 46, p. 290.

MALCOLM, P. (1932). Problems of Tuberculosis Control and its relation to Sanitary Science and Food Hygiene. *J. Amer. Vet. Med. Assocn.*, Vol. 80, p. 96.

MALOU, A. (1932). L'epidermo-reaction a la Tuberculine chez les animaux domestiques. Thesis for Doc. Vet., Alfort, p. 62.

MANTOUX (1908). Quoted by Hutyra, Marek and Manninger, p. 637, Vol. 1, 1938. Special Pathology Therapeutics of the Diseases of Domestic Animals.

MARSH, H., WARREN, D. M., AND MORROW, A. C. (1932). A study of the Tuberculin Sensitisation in cattle showing sub-cutaneous lesions. *Jnl. Amer. Vet. Med. Ass.*, Vol. 81, p. 105.

MINETT, F. C. (1932). Avian Tuberculosis in cattle in Great Britain. *J. Comp. Path. and Therap.*, Vol. 45, p. 317.

MINISTRY OF AGRICULTURE (BRITAIN) (1942). The Tuberculin test. *Vet. Rec.*, No. 20, p. 191.

MORO (1908). *Munchen. med. Wchnschr.*, Vol. 55, p. 217, quoted by Calmette. A. 1923.

MOUSSU, G. (1932). De l'emploi de la tuberculine dans le diagnostic de la tuberculose en police sanitaire. *C.R. Acad. Agric.*, Vol. 2, p. 54.

NOCARD (1891). *Bull. Acad. Med.*, Vol. 55, pp. 476-643, quoted by Calmette. A. 1923. Tubercle Bacillus infection and Tuberculosis in man and animals.

NOCARD (1892). *Ann de l'Inst. Pasteur*, Vol. 6, p. 44, quoted by Calmette. A. 1923. Tubercle Bacillus infection and Tuberculosis in man and animals.

PERARD, CH., AND RAMON, W. (1913). De l'Existence des Tuberculides chez les Bovides. *Compt. Rend. Soc. Biolog.* 133, a note presented by H. Vallee.

PLUM, N. (1926). *Cornell Vet.*, Vol. 16, p. 237.

PLUM, N. (1937). Control of Bovine Tuberculosis in Denmark *Skand. Vet. Tidskr.*, Vol. 27, p. 157, Summary in *Vet. Bulletin*, August 1938, Vol. 8, No. 8, p. 493.

POWER, W. M. (1927). Discussion on P. R. Viljoen's paper, Tuberculosis in S. Africa. *Jnl. of S.A.V.M.A.*, Vol. 1, No. 1, p. 20.

PRENDERGAST, W. M. (1936). Skin lesions in cattle. *Jnl. Amer. Vet. Med. Assocn.*, Vol. 88, p. 74.

ROBERTSON, A., AND HOLE, N. H. (1937). A preliminary report on the problem of the bovine skin lesion Tuberculin reactor. *Jnl. of Comp. Path and Therap.*, Vol. 50, No. 1.

RQECKL, AND SCHUETZ (1891). *Ztschr. F. Fleisch.* March, quoted by Calmette. A. 1923. Tubercle Bacillus infection and Tuberculosis in man and animals.

SCHROEDER, E. C. (1926). No lesion reactors. *Jnl. Amer. Vet. Med. Assocn.*, Vol. 99.

STENIUS, R. (1932). On Calipers for use in the Intradermal Tuberculin Test. *Skand. Vet., tidskr.*, Vol. 22, pp. 540-544. Summary in *Vet. Bulletin.*, September, 1933, Vol. 3, No. 9.

STENIUS, R. (1933). Notes on Technical details of the Intradermal Tuberculin Test. *Skand. Vet., tidskr.*, Vol. 23, pp. 102-109. Summary in *Vet. Bulletin*, September, 1933, Vol. 3, No. 9.

SAHAI, L. (1941). The interrelationship between the allergic responses of guinea pigs sensitised with various members of the acid-fast group of organisms. *Vet. Journal*, Vol. 97, No. 4, p. 113.

Sä VIANA CONTE, H., AND BELO, M. M. A. (1936). The intradermal Tuberculin Tests. *Rev. Med. Vet. Lisboa*, Vol. 31, pp. 48-61. Summary in the *Vet. Bulletin*, December, 1937, Vol. 7, No. 12.

SCHAEFER, W. (1935). Sur un type particulier de bacilles acido-resistants isoles de l'organisme de cobaye. *C.R. Soc. Biol.*, Paris, Vol. 119, p. 961.

SCHMIDT, AND HAUPT (1938). Quoted by Hutyra, Marek and Manninger. Special Pathology Therapeutics of the Diseases of Domestic Animals, 1938, p. 637, Vol. 1.

SPICER, J. (1933). Observations on the testing of cattle for Tuberculosis by the Double Intradermal method. *Vet. Record.* Vol. 13, p. 474.

SYMONDS, S. L. (1936). Notes on the application of the Tuberculin Test to dairy cows in a small area. *Aust. Vet. J.*, Vol. 12, p. 178.

TIMONEY, J. F. (1939). Avian tuberculosis in the Cow. *Vet. Record*, Vol. 51, Nos. 7 and 8.

TOPLEY, AND WILSON (1929). The Principles of Bacteriology and Immunity, Vol. 2.

TUTT, J. B. (1928). Personal communication to Buxton and MacNalty, the Intradermal Tuberculin Test in cattle, *Med. Research Council Special Report Series*, No. 122. London H.M. Stationery Office.

VAN PIRQUET (1907). *Deutsch. med. Wchnschr.*, Vol. 865, quoted by Calmette. A. 1923. Tubercle Bacillus infection and Tuberculosis in man and animals.

VAN HEELSBERGEN, T. (1932). Vogeltuberkulose by het Rund. *Tydschr. Diergeneesk.*, Vol. 59, p. 1093.

VILJOEN, P. R. (1927). Tuberculosis in S. Africa. *Jnl. of S.A.V.M.A.*, Vol. 1, No. 1, p. 20.

WIER, J. (1935). Remarks on the Double Intradermal Test. *Vet. Record*, Vol. 15, Nos. 1-4.

ZELLER, H. (1936). Beitrag zur Diagnose der Rindertuberkulose *Berl. tierarztl. Wschr.*, Vol. 52, p. 789.