THE DOUBLE INTRADERMAL TEST FOR TUBERCULOSIS IN CATTLE
THE DOUBLE TRADERMAL TEST FOR TUBERCULOSIS IN CATTLE, WITH REFERENCE TO THE "NO LESION" RE-ACTOR, NON-SPECIFIC REACTIONS AND PART PLAYED BY SAPROPHYTIC ACID FAST ORGANISMS IN CAUSING SENSATION TO BOVINE AND HUMAN TUBERCULIN

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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 (Topley and Wilson) 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 \( \frac{1}{10} \) 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 Guttmann (of Dorpat), Roeckl and Schuetz, Bang and Salomonsen, Lydtin, Bang, Nocard, Hutrya (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 or 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 m.m. long at intervals of a few centimeters. On two of these scarifications was placed and spread a drop of Koch's raw tuberculin/...
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 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 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 claims 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 in the eye. This test has not found 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 the Veterinary world as the Intradermalpalpebral test. A further modification of this test, the Intrapalpebral, was introduced by Favero and Finzi (Hutrya, Marek and Manninger). They injected 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 Jamuschke (Hutrya, 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 Intradermalpalpebral 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 and achieved great popularity. After having tried this method out extensively in Great Britain, a report was published in 1925 by Buxton and his co-workers 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 Test and is the prevailing one in most parts of the world. This test is preferred to the subcutaneous test although at times they are used in conjunction with each other.

TUBERCULOSIS TESTING IN SOUTH AFRICA. - Up to 1928 the Subcutaneous test was the method in vogue for the diagnosis
of tuberculosis in cattle and gave apparently 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 generally 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 with a very fair chance of success. As is recognised, however, neither this nor any biological test is 100% 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 reactor will usually be shown 24 hours after the first intradermal injection. Moussu and Mantoux recommended the subcaudal fold for the site of the injection, while Römer and Joseph 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 seats 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 as this is now a subcutaneous test. On the other hand, if the injection is made immediately under the surface epiteleum, swellings will occur/....
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 underlying skin, but it may also occur in hide-bound animals. In discussing injections made too deep, he also asserts that swellings may reach 1 inch 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 (1933) draws attention to the site of injection. He stresses the importance of not injecting too low down on the neck in case of the oedema in a reactor being 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 skins over the neck, 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 the site is contra-indicated. The writer in carrying out tests of many animals in the Union has never disinfected the skin.

Jorgenson (1936) is quoted in the Vet. Bulletin Vol. 7. No. 6. as stating that when tuberculin is injected in the deeper layers of the skin a stronger reaction developed than when injected more superficially. He further discusses the intensity of reactions in the intradermal test as used in Denmark. An analysis of 85 animals reactions were .22 m.m. greater when the injection was made behind the shoulder instead of in front of the shoulder. He experimented with varying doses of tuberculin (.05, .1, and .2 c.c.) for injection and showed that the largest dose caused the strongest reactions but stated that the smallest dose gave an unmistakable reaction.. Buxton and MacNalty (1928) recommend a dose of .1 c.c.. Buxton and Glover (1939) stress the fact that concentrated tuberculin in doses of .1 c.c. should be used for cattle owing to the low order of dermal sensitivity in these animals.


Various opinions are expressed about the measurement of the skin during testing. Tatt (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 do 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 /....
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 measurements increase in 39 non-tuberculous animals as 3 m.m. and for 39 tuberculous animals as 12.74 m.m. Finally they state that it was impossible to lay down any definite increase in skin measurement which could be taken as absolutely diagnostic of Tuberculosis.

Glover (1931) stated that the character of the swelling was of greater importance than the size. Green (1933) as a result of his experiences in carrying out the Tuberculin Test in Durban came to the conclusion that it was better to rely on the clinical character of the swelling than merely on the increase of skin measurement. He went so far as to state that after some experience it is better to discard the calipers altogether. Laenkholm (1934) summarised in the Vet. Bulletin Vol. 5, No. 9, 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) held the view that measuring the skin was not to be recommended. Douglas (1937) did not advise measuring the skin at the time of injection but at the 48th hour he measured 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) pointed out the bad points of the slide rule calipers 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 m.m., is considered too large. He introduced an improvised instrument which shows the readings on a circular dial with a moving pointer which measures up to a tenth of a millimeter.

Christiansen (1933) laid down that calipers must be accurate and graduated in half millimeters. In his opinion, in a herd believed to be free of Tuberculosis where the single intradermal test was carried out, an increase in measurement of the site of injection of 2.5 m.m.s. or over was indicative of a positive reactor. In other herds an increase of 3.5 m.m.s. or over in the skin measurement was classed as a positive reaction while an increase between 1.5 m.m. and 3.5 m.m.s. suggested a suspicious case.

Götzte and Muller (1936) describe a form of special calipers 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 up to within .1 m.m.

Zeller (1936) decided that an increase of 3 .m.m.s. in the skin measurements indicated a positive reactor and/...
and stated that on post mortem examination 96.8% 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) gave some views on what he termed a 'Pinch' and a 'Wipe' measurement and showed that there may be a difference of 4 m.m.s. or more in one swelling by these two methods. The possibility of obtaining such differences was due to his using calipers 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 was in favour of the 'pinch' measurement being adopted for interpreting the degree of swelling.

Lleavelyn Jones (1939) criticised Hancock on his suggestions regarding 'pinch and wipe' measurements and advised against using the compass type of caliper, preferring 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 Tuberculosis tests and states that apart from human errors or unsuitable Tuberculin, the test is 100% accurate. This can only be read as errors in interpretation of results. The Australian workers have marked views on interpretations. Henry (1936) stated that the disadvantages of the intradermal test were that results and the interpretation of the test rest so much on the skill and ability of the operator. Fethers, cited by Symonds (1936) pointed out that 'no lesion' reactors were 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 N.A.V.M.A. in December 1932, it was agreed in the main that the demonstration of heat and pain was necessary for the diagnosis of a positive reaction as well as local swelling.

Spicer (1932) laid stress upon the importance of recognising slight oedema in reactions. Green (1933) was of the opinion that a definite positive or definite negative reaction was easy to interpret and held that the cardinal symptoms of a positive reaction were a definite 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/....
these groups are of much use to men 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 in coming to a definite conclusion such as enlargement of the Prescapular gland on the side of the animal injected, systemic symptoms such as rigors, a high temperature, and a drop in the milk yield. The writer has also experienced cord ing of the lymphatic vessels close to the site of injection in some positive reacting animals. In conclusion Douglas (1937) holds that the character of the swelling is of much greater importance than its size.

SENSITISATION OF CATTLE TO TUBERCULIN.

In attempts to explain 'no lesion' reactors, many suggested explanations are brought forward. Schröeder (1926) expresses the view that undue importance is given to the question of the 'no lesion' reactor holding that the numbers of such reactors is negligible in comparison with the numbers 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). Schröeder goes further and states that the Federated Bureau of Animal Industries of the United Stated of America examined tissues from several thousand so-called 'no lesion' reactors and proved without doubt that about 25% 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 sensitise the animal tissues does not always of necessity result in or lead to tuberculous processes visible to the/....
the eye.

Stanious (1933) quotes statistics from Finland collected over a period of 10 years. These showed that about .1% of all cattle killed for food purposes were tuberculous, while on the other hand Tuberculin tests showed an incidence of positive reactors of 2 to 3%. Investigations carried out as a result of these findings showed that on several farms where Tuberculin tests gave weak positive reactions these cattle frequently belonged to 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) 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 to Tuberculin of either human or bovine type origin. 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 the inoculation of guinea-pigs with emulsions of the lymph glands M. tuberculosis (human type) was isolated from the lesions caused.

dekock (1932) reviewed different methods of Tuberculosis eradication, viz. Bang’s, ostertag’s, 1925 Tuberculosis order of Great Britain, and the accredited herd plan of America. Bang’s method suggested an annual tuberculin test while ostertag favoured Tuberculin tests every 3 months where cases had occurred. He advised that tests in South Africa should be carried out at 2 to 3 monthly intervals until two consecutive negative tests were 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) reported on this aspect of the Tuberculin test. Their conclusions were that the site of injection and for a distance of 2 to 4 inches from it showed an area of increased sensitivity for about 3 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 was pre-supposing that the re-test took place some few weeks after the original test.

in a personal communication, Jackson and Diesel (1939) state that in Natal a number of herds are under test for the eradication of Tuberculosis. In most of these herds all cattle have been submitted to testing on numerous occasions and the majority of them have given two consecutive negative tests. Recently
when the annual testing has taken place a number of cattle that have previously given negative reactions have suddenly shown swellings that simulate doubtful positive reactions. The increased measurement has sometimes been two and three times greater than the original skin fold figure, there has been slight pain and even a slight oedema. Re-testing has not assisted and on post-mortem examination of the more suspicious cases no visible lesions of tuberculosis have been found. Biological tests of glands from these cases have proved negative. No cases of open tuberculosis among owners or attendants have been observed. Newly introduced cattle onto these farms tested at the same time with the same batch of tuberculin have given clear cut negative reactions. A number of these doubtful reactors have been tested with Avian Tuberculin with definite 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.**

At the present time much attention is being focused 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 aborted. Post mortem examination revealed lesions in the uterus, the mesenteric lymph glands, the lung and in the bronchial gland. Bovine type of Tubercle bacilli were 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 as a result of 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) claimed that Avian Tuberculosis occurred much more commonly in bovines than was generally diagnosed. In such cases lesions were mainly confined to the lymph glands of the digestive and other organs.

In the report of the Chief, Bureau of Animal Industries, United States of America (1933) it was stated that evidence had 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 Schlott- hauer (1935) recount an experience with a herd of cattle that/......
that had been tested regularly for 11 years. Suddenly at a subsequent test 21 positive reactors were found. It was, however, 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 slaughtered small isolated lesions were found in the skin and mesenteric lymph glands. Material from 11 of these cases 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 that 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 but 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 was that the sensitisation 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 Avian Tuberculosis in cattle and states that in the great majority of these cases they were 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 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 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 to intradermal tuberculin tests when bovine tuberculin is used. He came to the conclusion that one of the characteristics of avian type of infection was the absence of any demonstrable naked eye lesions although infection was present in the tissues. Such animals would 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 by 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) drew attention to the fact that in South Africa little or no attention had been paid to the presence or absence of skin lesions in tuberculin reacting cattle and stated that in the United States of America this...