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COMPARATIVE INTRADERMAL TESTS BETWEEN CONCENTRATED P.P.D. AND DILUTED P.P.D. TUBERCULINS IN RELATION TO NON-SPECIFIC TUBERCULIN REACTORS.

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The British Ministry of Agriculture (1947) introduced the single intradermal comparative test for all official tuberculin tests as from the 2nd June 1947. The tuberculin for this single test is used in a concentration of 3 mg. per c.c. P.P.D. mammalian and 0·8 mg. per c.c. avian. Full details are given as to dosage (0·1 c.c. and reading the reaction at the 72nd hour) and how to interpret the readings obtained.

Fourie (1952) in an article handed in for publication in October, 1950. expressed the opinion that tuberculin in a concentration of 3 mg. per c.c. is too potent and is inclined to give too many non-specific reactions. He suggested that for the single test the British Ministry's retest tuberculin, viz., 1·5 mg. per c.c., which is the same strength as that which has in the past been so successfully used for the double intradermal test, should also be used for the single test.

At the annual tuberculin test (1950) using P.P.D. Onderstepoort tuberculin 3 mg. per c.c. as a single test on some 760 head of cattle belonging to the Division of Veterinary Services and running on the farms Armoedsvlakte and Biesiesvlakte in the Vryburg District, 9·4 per cent of the animals reacted by considerably increased measurements. However, the nature of the swellings was such, that they were not regarded as true positive reactions, but were interpreted as non-specific reactions. On this account this herd was considered to be very suitable for making a comparative test of the tuberculin in concentrations of 3 and 1·5 mg. per c.c. respectively.

In order to make sure if the reactions obtained in September, 1950, were non-specific reactions, the short thermal tuberculin test as used by Gregory (1949) was applied to 5 of some of the worst reactors together with a negative control (8920). These results are presented in Table No. 2. Details of the single intradermal test (September 1949) and the comparative test on these 6 animals (3 mg. and 1·5 mg. per c.c.—November 1950) are presented in a combined Table No. 1.

From this table it is seen that 3 out of 5 animals reacted negatively to both the 3 mg. and the 1·5 mg. per c.c. tuberculin. Of the remaining two 688 and 3227, sensitization was still present, but on a lower level than was the case in September, 1950. In the case of 688, there was really no difference in the reactions produced by the 3 and the 1·5 mg. per c.c., but in the case of 3227 the reactions to the 3 mg. per c.c. was definitely greater.

The short thermal tuberculin test Gregory (1949) was applied to the 6 animals referred to in Table No. 1 on the 15th November, 1950, i.e. at the same time as the comparative test was being applied to the two herds (see Table No. 2).

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TABLE NO. 1.

Combined Table giving Details of Single Intradermal Test 3 *mg. per c.c. September,* 1950 *and of Comparative Single Test* 3 *mg. and* 1·5 *mg. per c.c. November* 1950 *on* 5 *of some of the Worst September,* 1950 *Reactors with Negative Control* 8920.

No. of Animal.	17–19 September, 1950.			16–19 November, 1950.				
	3 mg. P.P.D. per c.c.			Left Side 3 mg. P.P.D. per c.c.		Right Side 1·5 mg. P.P.D. per c.c.		Result.
688	8·0	17·0	Retest.	8·0	12·6	8·0	12·0	Retest.
3192	6·0	9·2	Retest.	6·3	6·5	6·3	6·9	Neg.
3227	14·5	24·0	Retest.	13·9	22·0	14·1	18·0	Retest.
3330	6·5	11·0	Retest.	6·5	7·0	7·4	7·9	Neg.
8916	10·5	21·0	Retest.	10·0	10·0	10·0	10·0	Neg.
8920	9·0	9·0	Neg.	9·0	10·0	8·8	9·0	Neg.

TABLE NO. 2.

Short Thermal Tuberculin Test on the 6 *Animals referred to in Table No.* 1.

No. of Animal.	Pre-injection Temp.		Post Injection Temperatures.					Result.
	14/11/50.	15/11/50, 9 a.m.	10 a.m.	12 noon.	2 p.m.	3 p.m.	4 p.m.	
688	102·8	100·6	100·4	100·6	100·6	101·2	101·2	Neg.
3192	103·8	100·4	100·8	100·4	101·6	101·8	101·8	Neg.
3227	100·6	100·6	100·4	100·0	100·2	100·6	100·4	Neg.
3228	102·6	100·8	101·4	100·6	100·8	101·2	101·6	Neg.
3330	102·2	100·6	100·4	100·4	101·0	101·2	101·4	Neg.
8916	104·0	101·2	101·4	101·4	102·0	102·6	102·4	Neg.
8920	103·2	100·8	100·4	100·4	101·2	101·4	101·6	Neg.

The results of this test were completely negative. This is further confirmatory evidence that the reactions were non-specific, in this sense that they were not due to the effects of the bovine type of organism.

The high first pre-injection temperature recorded for a few animals, was due to the fact that those temperatures were taken not very long after the animals were brought in from the veld. The animals were then kept in a paddock close to the laboratory and the pre-injection temperatures the next morning were normal.

Comparative Intradermal Test Using 3 *mg. and* 1·5 *mg. per c.c. Onderstepoort P.P.D. Tuberculin on a Herd which was free of Tuberculosis for a Number of Years.*

As already explained the two herds at Armoedsvlakte and Biesiesvlakte, comprising 760 animals, were used. From the available evidence it was concluded, that the sensitization which was present in quite a number of individuals in the two herds, was due to non-specific factors, or more precisely was not due to the effects of the bovine type of organism.

Tuberculin at a concentration of 3 mg. per c.c. was injected into the skin on the left side of the neck and at a concentration of 1·5 mg. per c.c. on the right side of the neck by a different worker. The measurements of the reactions on both the left and right side of the neck were made by the same worker.

It is not considered necessary to reproduce the figures obtained from each of the 760 animals, but a summary of the statistical analysis is reproduced in Table 3.

TABLE No. 3.

	No. of Animals.	Percentage Reactors in Relation to Total Retested.
Reactors to 3 mg. per c.c. only.....	70	9·2
Reactors to 1·5 mg. per c.c. only.....	41	5·4
Reactors to 3 and 1·5 mg. per c.c. at same time.....	73	9·6
TOTAL NUMBER OF REACTORS.....	184	24·2
TOTAL NUMBER OF ANIMALS TESTED.....	760	—

From the above it will be seen that of the 760 animals tested, 73 reacted to both the 3 mgm. and the 1·5 mgm. concentration per c.c., 70 reacted only to the 3 mgm. and 41 only to the 1·5 mgm. concentration.

The difference in the number of reactors (70) produced when using 3 mg. per c.c. P.P.D. Onderstepoort tuberculin as compared with the number (41) produced by 1·5 mg. per c.c. is statistically highly significant in favour of the 3 mg. per c.c. concentration.

Put in another way, there were altogether 184 animals which showed reactions. Of these 143 (77·7 per cent) reacted to 3 mg. per c.c. and 114 (62 per cent) reacted to 1·5 mg. per c.c. The difference of 29 (15·7 per cent) is also considered to be highly significant statistically.

This being the case, the 1·5 mg. per c.c. should be the concentration of choice, if it will pick out positive reactors as accurately as the 3 mg. per c.c. Accordingly arrangements were made to make a comparative test with the two concentrations in question on a positive herd.

Comparative Intradermal Test Using 3 mg. and 1·5 mg. per c.c. Onderstepoort P.P.D. Tuberculin on a Positive Herd.

Sixty two animals which were at various times previously declared positive reactors by the intradermal test, were used. They were divided into two groups of 27 animals in one and 35 animals in the other. In the case of the 1st group 3 mg. per c.c. Onderstepoort P.P.D. tuberculin were injected into the skin on the left side of the neck and 1·5 mg. per c.c. into the skin at the corresponding spot on the right side.

The same procedure was followed in the case of the 35 animals in the 2nd group, excepting that now the 1·5 mg. per c.c. were injected on the left side and 3 mg. on the right side (see Tables 4 and 5).

TABLE 4.

Group I.

Name.	Dose and Site of Injection.	Normal Skin.	72 Hours.	Results.
1. Angelier	3 mgm. per c.c., left	5·0	12·5	R.
	1·5 mgm. per c.c., right	5·0	10·0	R.
2. Grietjie	3 mgm. per c.c., left	9·0	17·0	P.
	1·5 mgm. per c.c., right	9·0	14·6	P.
3. Leeutjie	3 mgm. per c.c., left	6·0	18·5	P.
	1·5 mgm. per c.c., right	8·0	16·0	P.
4. Ounooi	3 mgm. per c.c., left	8·0	10·0	N.
	1·5 mgm. per c.c., right	8·0	10·0	N.
5. Alberta	3 mgm. per c.c., left	7·0	17·0	P.
	1·5 mgm. per c.c., right	7·0	14·5	P.
6. Anna-Marie	3 mgm. per c.c., left	5·0	15·0	P.
	1·5 mgm. per c.c., right	5·0	11·0	P.
7. Engeltjie	3 mgm. per c.c., left	7·0	12·5	P.
	1·5 mgm. per c.c., right	7·0	10·8	R.
8. Sonstraal	3 mgm. per c.c., left	7·0	18·9	P.
	1·5 mgm. per c.c., right	7·0	17·2	P.
9. Lovely	3 mgm. per c.c., left	5·0	17·5	P.
	1·5 mgm. per c.c., right	5·0	15·0	P.
10. Orpa	3 mgm. per c.c., left	5·0	12·0	P.
	1·5 mgm. per c.c., right	5·0	11·0	P.
11. Katoo	3 mgm. per c.c., left	9·0	23·5	P.
	1·5 mgm. per c.c., right	8·0	17·0	P.
12. Christa	3 mgm. per c.c., left	6·0	14·2	P.
	1·5 mgm. per c.c., right	6·0	12·5	P.
13. Annelisa	3 mgm. per c.c., left	7·0	10·0	N.
	1·5 mgm. per c.c., right	6·0	7·5	N.
14. Rachel	3 mgm. per c.c., left	6·0	7·5	N.
	1·5 mgm. per c.c., right	6·0	6·5	N.
15. Magdalena	3 mgm. per c.c., left	5·0	30·6	P.
	1·5 mgm. per c.c., right	6·0	26·0	P.
16. Seigis	3 mgm. per c.c., left	6·0	19·5	P.
	1·5 mgm. per c.c., right	7·0	18·5	P.
17. Minerva	3 mgm. per c.c., left	7·0	18·5	P.
	1·5 mgm. per c.c., right	8·0	12·8	R.
18. Lulu	3 mgm. per c.c., left	7·0	15·0	P.
	1·5 mgm. per c.c., right	8·0	10·5	R.
19. Melkbron	3 mgm. per c.c., left	8·0	22·6	P.
	1·5 mgm. per c.c., right	8·0	17·2	P.
20. Siebeltjie	3 mgm. per c.c., left	6·0	17·0	P.
	1·5 mgm. per c.c., right	6·0	13·8	P.
21. Rene	3 mgm. per c.c., left	6·0	11·2	R.
	1·5 mgm. per c.c., right	8·0	9·5	N.
22. Angela	3 mgm. per c.c., left	6·0	18·5	P.
	1·5 mgm. per c.c., right	8·0	16·0	P.
23. Rintjie	3 mgm. per c.c., left	6·0	13·8	P.
	1·5 mgm. per c.c., right	6·0	14·5	P.
24. Peggy	3 mgm. per c.c., left	6·0	24·0	P.
	1·5 mgm. per c.c., right	6·0	23·5	P.
25. Rachel II	3 mgm. per c.c., left	5·0	16·6	P.
	1·5 mgm. per c.c., right	5·0	12·0	P.
26. Matilda	3 mgm. per c.c., left	6·8	16·5	P.
	1·5 mgm. per c.c., right	6·0	10·5	R.
27. Antjies	3 mgm. per c.c., left	6·0	11·0	R.
	1·5 mgm. per c.c., right	6·0	9·0	R.

TABLE 5.

Group II.

Name.	Dose and Site of Injection.	Normal Skin.	72 Hours.	Results.
28. Lilian	1·5 mgm., left	5·0	5·5	N.
	3·0 mgm., right	5·0	8·5	N.
29. Suster	1·5 mgm., left	7·0	15·4	P.
	3·0 mgm., right	7·0	15·4	P.
30. Lady Eva	1·5 mgm., left	7·0	14·0	P.
	3·0 mgm., right	8·0	19·0	P.
31. Morelig	1·5 mgm., left	5·0	6·0	N.
	3·0 mgm., right	6·0	8·5	N.
32. Milly	1·5 mgm., left	5·0	8·5	N.
	3·0 mgm., right	6·0	17·2	P.
33. Elsie	1·5 mgm., left	6·0	12·5	R.
	3·0 mgm., right	7·0	13·8	R.
34. Angelic	1·5 mgm., left	6·0	10·0	R.
	3·0 mgm., right	6·0	11·8	R.
35. Susje	1·5 mgm., left	6·0	10·0	R.
	3·0 mgm., right	6·0	12·8	R.
36. Oleander	1·5 mgm., left	5·0	14·0	P.
	3·0 mgm., right	5·0	16·5	P.
37. Celia	1·5 mgm., left	5·0	16·5	P.
	3·0 mgm., right	5·0	16·2	P.
38. Aggie	1·5 mgm., left	7·0	27·6	P.
	3·0 mgm., right	7·0	30·0	P.
39. Sarie	1·5 mgm., left	6·0	7·0	N.
	3·0 mgm., right	6·0	7·0	N.
40. Suzan	1·5 mgm., left	5·0	11·2	R.
	3·0 mgm., right	7·0	10·5	R.
41. Aaltjie	1·5 mgm., left	5·0	12·5	P.
	3·0 mgm., right	6·0	14·0	P.
42. Sonneblom	1·5 mgm., left	8·0	15·2	P.
	3·0 mgm., right	8·0	15·5	P.
43. Ouma	1·5 mgm., left	5·0	8·9	R.
	3·0 mgm., right	5·0	9·0	R.
44. Ancy	1·5 mgm., left	6·0	8·0	N.
	3·0 mgm., right	6·0	7·5	N.
45. Rose-marie	1·5 mgm., left	9·0	12·4	N.
	3·0 mgm., right	9·0	12·2	N.
46. Maud	1·5 mgm., left	6·0	18·2	P.
	3·0 mgm., right	7·0	22·0	P.
47. Marina	1·5 mgm., left	7·0	22·0	P.
	3·0 mgm., right	5·0	25·0	P.
48. Lenie	1·5 mgm., left	5·0	21·5	P.
	3·0 mgm., right	6·0	22·0	P.
49. Racine	1·5 mgm., left	5·0	14·8	P.
	3·0 mgm., right	7·0	17·0	P.
50. Georgina	1·5 mgm., left	6·0	24·8	P.
	3·0 mgm., right	7·0	30·5	P.
51. Ada	1·5 mgm., left	7·0	14·2	P.
	3·0 mgm., right	7·0	12·5	P.
52. Albertjie	1·5 mgm., left	7·0	11·8	R.
	3·0 mgm., right	7·0	13·5	P.
53. Anna	1·5 mgm., left	6·0	11·5	R.
	3·0 mgm., right	6·0	15·0	P.
54. Rintjie	1·5 mgm., left	6·0	6·4	N.
	3·0 mgm., right	6·0	7·4	N.
55. Sanna	1·5 mgm., left	6·0	8·4	N.
	3·0 mgm., right	6·0	8·9	N.

TABLE 5 (*continued*).

Name.	Dose and Site of Injection.	Normal Skin.	72 Hours.	Results.
56. Erica	1·5 mgm., left	5·0	9·6	R.
	3·0 mgm., right	5·0	10·0	R.
57. Stella	1·5 mgm., left	5·5	9·5	R.
	3·0 mgm., right	5·0	7·8	N.
58. Loela	1·5 mgm., left	7·0	11·0	R.
	3·0 mgm., right	8·0	13·0	P.
59. Melissa	1·5 mgm., left	6·0	8·0	N.
	3·0 mgm., right	6·0	10·0	R.
60. Estelle	1·5 mgm., left	6·0	13·8	P.
	3·0 mgm., right	8·0	15·0	P.
61. Anita	1·5 mgm., left	5·0	5·5	N.
	3·0 mgm., right	5·0	6·0	N.
62. Violet	1·5 mgm., left	6·0	7·4	N.
	3·0 mgm., right	6·0	8·5	N.

In interpreting the test, both the size and the nature of the reactions were carefully considered, but there is no doubt that mistakes in interpretation were made. For instance the cow Albertjie No. 52 was at the time known to be a case of tuberculosis, actually excreting tubercle bacilli in her milk. According to the nature of the swelling she was classified as a "Retest" with the 1·5 mgm. concentration, but just made the positive classification with the 3 mgm. strength. Other cases have been encountered where so-called dead swellings were classified as positive to the test, but found to be no-lesion reactors on post mortem examination and others again where such dead swellings were produced by the intradermal test, but lesions of tuberculosis were found on post mortem examination.

It is also an established fact, that although these animals all had given positive reactions, some of them which later died or were slaughtered, were carefully examined and no lesions could be found.

In the human subject Palmer, Ferebee and Petersen (1950) found sensitization to what they refer to as high doses of tuberculin in a limited geographic region, in America. This they consider as evidence which suggests "the existence of a local wide-spread non-specific sensitivity to tuberculin, possibly caused by infection by an organism allergenically related to the tubercle bacillus".

Admittedly many so-called no-lesion reactors are actually infected animals. To quote only one case referred to by Biester & Murray (1928), who after making a most painstaking post mortem examination and cutting the organs and glands into ribbons, eventually concentrated on the only abnormality they could find viz. two moderately enlarged and reddened mesenteric lymphatic glands in the jejunal region. These glands were divided into two nearly equal portions. One portion was used for histological examination and the other for biological examination. This division was done by cutting the glands into fine sections and by taking alternate sections for histological and biological examination. The biological examination which is generally regarded as the most reliable method of demonstrating the presence of tubercle bacilli was negative.

Smears from the cut surface of the glands were made even though no macroscopic lesions were seen and after 3 hours search some acid fast organisms were found.

In cutting one of the embedded blocks, a very fine pin-point area drew attention and on histological examination, very early small lesions of tuberculosis were found. Three days were spent on this part of the examination.

It was not merely luck but also persistent effort which resulted in showing that this was not a no-lesion reactor, but it is clearly not practical to spend so much time on each case.

Although the owner's primary object in maintaining this positive herd was to build up his negative herd and to maintain some important blood lines, he did from time to time slaughter those animals showing signs of generalization. Undoubtedly animals with generalization were present at the time of the above test, since there is no method of recognising generalization immediately it takes place. For this reason, this herd was perhaps not so suitable for the above test, since animals in any state of desensitization, constitute a problem in tuberculin testing, even where a very potent tuberculin is being used.

The results of this comparative test presented in Tables 4 and 5 are summarised in Table 6 below.

TABLE 6.

A Summary of the Results in Tables 4 *and* 5.

Group.	Positive.	Retest.	Negative.	Total.
Gr. I—3 mgm., left	21	3	3	27
Gr. II—3 mgm., right	18	7	10	35
TOTAL 3 MGM., RIGHT AND LEFT...	39	10	13	62
Gr. I—1·5 mgm., right	17	6	4	27
Gr. II—1·5 mgm., left	14	10	11	35
TOTAL 1·5 MGM., RIGHT AND LEFT.	31	16	15	62

If the summarised results in Table 6 are studied, it will be seen that more positives were found with the 3 mgm. strength (39) than with the 1·5 mgm. strength (31), but in the case of the retests, there were more with the 1·5 mgm. strength (16) than with the 3 mgm. concentration (10).

It is a great pity that it was not possible to control these reactions with full post mortem examinations, but the animals were much too valuable from a breeding point of view, to be sacrificed in this manner. An attempt was, however, made to control some of these reactions by the short thermal tuberculin test of Gregory (1949). This test was carried out on the 15th August, 1951, details of which are presented in Table No. 7.

Only 19 out of the 62 animals could be tested and these fell into 4 groups.

(*a*) Five animals which gave positive reactions to both the 1·5 and the 3 mgm. concentration. They are Grietjie (2), Anna-Marie (6), Orpa (10), Christa (12) and Marina (47).

Gregory's test confirmed these positive intradermal reactions, except in the case of Orpa. In this animal there was a rise of temperature, but not to the recognised level for a positive reaction (104° F.). The

rise in temperature, however, was such that it could not be regarded as negative, but as a retest and for all practical purposes also a positive reactor (103·8).

(*b*) One animal, Morelig (31), which gave negative reactions to both concentrations and which is now also negative to the Gregory test.

(*c*) Six animals, which gave retest reactions to both the 3 mgm. and 1·5 mgm. concentrations. They are: Elsie (33), Angelic (34), Susje (35), Suzan (40), Ouma (43) and Erica (56). The Gregory test in all these cases is negative.

(*d*) Five animals which were positive to the 3 mgm. concentration and retest to the 1·5 mgm. concentration. They are: Engeltjie (7), Lulu (18), Albertjie (52), Anna (53) and Loela (58).

The positive reaction was confirmed in Engeltjie (7) and Lulu (18), negative in two and retest in Albertjie (52) which was known to excrete tubercle bacilli in her milk.

(*e*) A miscellaneous group of 2 animals: Stella (57) (retest to 1·5 and negative to 3 mgm.), she is negative to the Gregory test and Melissa (59) (negative to 1·5 and retest to 3 mgm.), she is also negative to the Gregory test.

TABLE 7.

Short thermal subcutaneous test of Gregory on 19 *of the* 62 *animals in the positive herd* (*see Tables* 4 *and* 5).

No.	Name.	Pre-injection. 7.30	2 Hrs. 9.30	4 Hrs. 11.30	6 Hrs. 1.30	7 Hrs. 2.30	8 Hrs. 3.30	10 Hrs. 5.30	11 Hrs. 6.30	Result.
2	Grietjie.....	101·3	102·0	102·2	102·7	103·0	104·2	105·5	—	Pos.
6	Anne-Marie..	102·2	103·0	102·6	104·0	104·0	104·6	104·6	—	Pos.
7	Engeltjie.....	101·3	102·0	102·2	102·7	103·0	104·2	105·6	—	Pos.
10	Orpa.....	101·5	101·9	101·4	102·6	102·6	103·0	103·8	103·0	Retest.
12	Christa.....	102·0	102·0	102·3	102·2	103·2	104·4	105·8	—	Pos.
18	Lulu.....	101·9	102·1	101·9	102·2	102·5	102·8	103·8	105·0	Pos.
31	Morelig.....	101·1	101·1	101·4	101·6	101·6	101·6	101·6	—	Neg.
33	Elsie.....	101·8	101·5	101·4	102·0	101·3	102·2	101·8	—	Neg.
34	Angelic.....	102·6	102·2	103·2	102·5	102·0	103·0	102·6	—	Neg.
35	Susje.....	102·2	101·2	101·6	102·5	102·8	102·5	102·0	—	Neg.
40	Suzan.....	102·3	102·8	101·6	102·3	102·5	102·4	102·5	—	Neg.
43	Ouma.....	101·0	101·2	101·0	101·8	101·8	102·0	102·0	—	Neg.
47	Marina.....	101·4	101·6	100·8	101·5	101·5	101·7	103·2	104·8	Pos.
52	Albertjie.....	101·6	102·1	101·6	101·8	102·4	103·0	103·2	102·4	Retest.
53	Anna.....	101·9	100·4	101·2	102·2	102·3	102·2	102·8	—	Neg.
56	Erica	102·0	101·6	102·0	102·3	102·2	102·6	103·0	102·8	Neg.
57	Stella.....	102·0	101·7	101·6	101·8	102·4	103·0	102·4	—	Neg.
58	Loela.....	101·3	100·2	101·2	101·4	101·7	102·0	102·0	—	Neg.
59	Melissa.....	101·8	101·5	102·3	102·2	102·5	102·5	102·7	—	Neg.

The results of the test with the two concentrations are no better or worse in the case of groups a, b, c and e, i.e. in 14 out of the 19 animals, but in the five animals in group (*d*) (positive to 3 mgm. intradermal and retest to 1·5 mgm. intradermal) the Gregory test is positive in two, retest in one (Albertjie known to be infected) and negative in the remaining two.

At the time the owner should have been asked to allow a post mortem examination on the two Gregory negatives (Anna and Loela) in order to make sure if they were free of infection. Unfortunately their importance was only realised when the results were carefully analysed months later and as they could in the meantime have become infected from contact with other infected animals, one would in the case of a positive post mortem not have been able to say that they were not infected at the time of the test.

If they were not false positives then naturally it would be in the interest of tuberculosis eradication to deal with them as well as with the two positives (confirmed by Gregory) as positive reactors with as little delay as possible. On the other hand, if they were false positives to 3 mgm. and on retest shown to be negative, one would not unnecessarily have sacrificed two valuable animals.

In cases of generalization with partial desensitization, it is true that one may possibly get negatives with the 1·5 mgm. concentration, whereas the 3 mgm. concentration may show a retest reaction, as a result of which one may eventually recognise the true state of affairs and prevent the further spread of the disease. When dealing with animals in a state of partial desensitization, one can hardly use a tuberculin which is too potent and it may not be good practice to use 1·5 mgm. strength in a herd, where it is possible that animals with generalization may be present.

A much better opportunity to test out concentrated P.P.D. tuberculin against dilutions of this tuberculin was afforded by a herd in the Vryheid District, Natal, where at the last test 18 animals with doubtful reactions were found and at the time it was thought that these might be non-specific reactors.

At the retest of these animals three months later it was decided to make a special attempt to make sure if the reactions were in fact non-specific and to try to establish the cause of such reactions, by slaughtering some animals and making a detailed post mortem examination.

At the retest all 18 animals were injected intradermally on the one side of the neck, with the standard concentrated tuberculin P.P.D. now being made at Onderstepoort at a concentration of 2 mgm. per c.c. Ten of these animals were injected intradermally at the corresponding spot on the other side of the neck with a 50 per cent dilution of this concentration, whilst the remaining eight were similarly injected with a 20 per cent dilution.

At the 72nd hour all 18 animals were to have been submitted to the short thermal subcutaneous test of Gregory. Actually only 16 could be used for this test.

Details of these tests are presented in a combined Table No. 8, in which is also given the results of the previous intradermal tests with P.P.D. Onderstepoort tuberculin carried out by the State Veterinary Officers concerned.

From the results of these tests as presented in Table 8, it will be seen that:—

(1) All 18 animals now gave full positive reactions to the concentrated P.P.D. tuberculin (2 mgm. per c.c.) except the old cow Very Nice (21) which again gave a retest reaction, although in her case the nature of the reaction, with the diffuse thickening of the skin was thought to be significant and reminded one of the same kind of reaction one sees in cases of generalization with partial desensitization.

TABLE 8.

No.	Name.	Test 24–26/10/51.		Test 31–2/2/52.		Test 9–11/6/52, 2 mgm. per c.c., 0 Hrs.	P.P.D. Conc. 2mgm. 72 Hrs.	P.P.D. 50% 72 Hrs.	P.P.D. 20% 72 Hrs.	Remarks.	Short Thermal Subcutaneous Test—Gregory.						Results Gregory Test.
		0 Hrs.	72 Hrs.	0 Hrs.	72 Hrs.						10 a.m. Pre-injection	12 noon. 2 Hrs.	2 p.m. 4 Hrs.	4 p.m. 6 Hrs.	5 p.m. 7 Hrs.	6 p.m. 8 Hrs.	
17	Happy.....	5·0	11·5	7·0	14·0	5·0	19·5	—	14·5	Pos.....	100·6		Not t	ested	Gregor	y	Positive.
18	Witbors...	4·5	11·0	6·0	11·0	4·5	20·5	14·5	—	Pos.....	101·5	102·4	103·4	104·4	104·9	105	Positive.
20	Pikanien...	4·0	10·0	4·0	9·0	4·0	17·5	17·0	—	Pos.....	101	101·2	101·6	103·8	104	105·6	Positive.
21	Very Nice.	5·0	12·5	5·0	12·0	4·0	11·0	—	8·5	Dif. thick Retest / Dif. thick Retest	101·4	102	103·6	104·2	103·6	104	Positive.
25	Poppy.....	3·0	8·0	4·0	11·0	4·0	16·0	10·0	—	Dif. thick Pos. / Retest	101·2	102·6	102·6	103·2	104·3	105·5	Positive.
27	Strikkie....	4·5	8·0	4·0	10·0	3·5	12·0	—	11·5	Dif. thick Pos. / Dif. thick Pos.	102	102·4	102·8	103·3	103·6	104	Positive.
30	Bontrok...	4·0	14·5	4·0	13·5	4·0	26·0	—	20·5	Pos.....	102·2	102·2	102	103·0	103	104	Positive.
31	Skiller.....	4·5	11·5	6·0	16·5	4·5	22·0	—	19·0	Pos.....	102	102·2	102·4	105·8	106·4	106·9	Positive.
32	Tweespeen	5·0	9·0	7·0	14·0	5·0	25·5	18·5	—	Pos.....	101·8	102·2	102·8	104·8	105·6	106	Positive.
34	Mooimeid.	5·0	11·5	5·0	13·0	5·0	25·0	—	23·5	Pos.....	101·8		Not	tested	Gregor	y	Positive.
35	Strikjan...	4·0	9·0	4·0	10·0	4·0	13·0	9·5	—	Pos./Retest..	101·2	102	101·8	103·4	103·8	105	Positive.
36	Dopey.....	5·0	11·0	6·0	14·0	5·0	19·0	—	15·0	Pos.....	101·6	102	101·8	103·5	104·2	105·2	Positive.
37	Rooimier..	4·0	8·0	6·0	12·5	4·0	16·0	12·0	—	Pos.....	102·8	102·4	102	105·4	105·8	106·4	Positive.
41	Wit.....	4·5	9·0	4·0	9·0	5·0	17·0	11·0	—	Pos./Retest..	100	102·5	104	105·8	106	106·8	Positive.
45	Poplap....	5·0	12·5	5·5	13·0	5·5	23·0	19·0	—	Pos.....	101·6	102·2	102·4	104·1	104·6	105·4	Positive.
46	Windvoël..	4·5	11·5	4·0	9·0	4·0	22·0	—	14·0	Pos.....	102	101·8	102·8	102·8	103·1	106	Positive.
55	Majie.....	4·5	10·0	5·0	11·5	6·0	26·5	20·0	—	Pos.....	101·4	101·8	102·2	103·7	104	104·6	Positive.
72	Witbeen...	4·0	10·0	5·0	11·0	4·5	18·0	21·0	—	Pos.....	99	102·2	103	105·4	105·6	105·8	Positive.

(2) The eight animals tested with the 20 per cent P.P.D. including the old cow Very Nice gave exactly the same end result as the 2 mgm. per c.c. concentration, but in all cases the swellings were smaller.

(3) The 10 animals tested with the 50 per cent tuberculin (1 mgm. per c.c.) gave full positive reactions in 7 and retest reactions in three [Poppy (25), Strikjan (35) and Wit (41)], whereas these three animals gave positive reactions with the full strength tuberculin. Again the size of the swellings was smaller, excepting in Witbeen (72) where the swelling was unexpectedly larger.

Actually one could have interpreted the three retests with the 50 per cent tuberculin, also as positives, the measurements being 4·0—10·0, 4·0—9·5 and 5·0—11·0, but since the swellings were a good deal smaller than those with the full concentration and in trying to judge the reactions as objectively as possible, it was decided to interpret them as retests, especially as in the case of Strikjan, the swelling was definitely circumscribed.

(4) The short thermal subcutaneous test was positive in all the 16 animals that could be tested including the old cow Very Nice, which was considered to be a retest with the 100 per cent and the 20 per cent P.P.D. tuberculins intradermally.

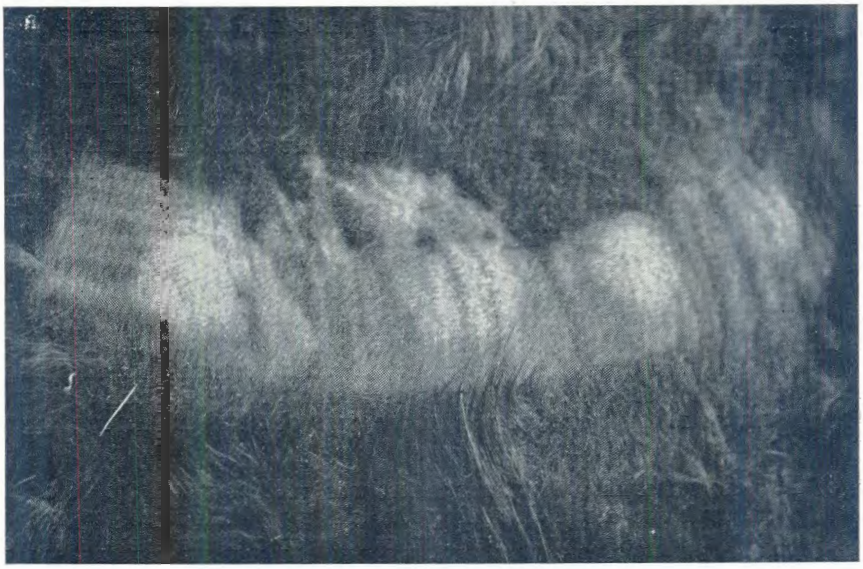
Although the old cow Very Nice gave the smallest intradermal reactions and was probably wrongly designated as a retest, even on those measurements, the nature of the reaction was such, that she was regarded as a case of generalization with partial desensitization especially also in view of the positive short thermal subcutaneous test. For this reason the owner, who was in any case anxious to see the results of the tests confirmed by post mortem examination on at least one animal, was asked to destroy this old cow. This was accordingly done and generalised tuberculosis was found to be present.

The reason why these animals now gave positive reactions, whilst three months earlier they gave retest reactions, is probably due to a recent infection say four to five months before the last test. As a result of such recent infection they had not yet developed the normal sensitization at the first test, but did so three months later at the last test.

General Discussion.

The results of the tests on these 18 animals indicate quite clearly that even a very dilute tuberculin will produce a type of intradermal reaction that can be read with reasonable confidence, if there is what can be described as normal sensitization.

It would further appear that the size of the reacting swelling and not so much the nature of this swelling is, if not directly proportional, at least very closely related to the potency of the tuberculin used. This is clearly seen in the accompanying photomicrograph showing the reaction of an animal artificially infected with a known virulent bovine type of tubercle bacillus when tested with (1) normal standard Onderstepoort P.P.D. tuberculin (2 mgm. per c.c.), (2) 50 per cent normal standard. (3) 20 per cent normal standard, and (4) 10 per cent normal standard.



Infected bovine with normal sensitization tested with 2 mgm. per c.c. P.P.D., 50%, 20%, and 10% P.P.D. of 2 mgm. per c.c. concentration.

The actual measurements recorded at the time are:—

Normal skin.	2 mgm. per c.c.	50%	20%	10%
7·0	22·0	22·0	20·0	16·0

Visual inspection reveals the difference in the reactions with the diluted tuberculin much better than the actual measurements.

From the available evidence as presented in this paper, it would appear that one is able to pick out reactors in a normal state of sensitization, with a very much more dilute tuberculin than is ordinarily used.

Since the 1·5 mgm. per c.c. strength has for many years given such excellent results in the double intradermal test, it is suggested, that this strength be also used in the single test. It will probably not be found to be very much inferior to the 3 mgm. strength in picking out cases of generalization with partial desensitization, if not as full positive reactors, then at least as retest reactors.

In using the 1·5 mgm. strength one is less likely to find so many false positives as would be the case with the 3 mgm. concentration. Further work in the light of the above may show that an even more dilute tuberculin may be the concentration of choice, especially in the annual tests of herds in which the disease had been eradicated, but which may at any time again become re-infected. In such cases one is unlikely to meet with animals having a subnormal sensitization and there is less chance of interpreting non-specific reactions as false positives.

The above recommendations would have been made with a much greater degree of confidence if comparative tests with dilute tuberculin could have been carried out on a greater number of positives controlled by post mortem examina-

tion and if one had the opportunity of making similar tests on those specific non-specific cases in which positive evidence of some degree of sensitization to tuberculin had been established. Unfortunately such cases of Johne's Disease, Brucellosis, avian and human type infection, actinomycosis, skin lesions and abscesses were not available for testing. A case seems nevertheless to have been made out for the necessity of paying much more attention to the optimum concentration of tuberculin for the single intradermal test.

SUMMARY.

1. In a herd of 760 animals which has for years been more or less regularly tuberculin-tested and in which for years no case of tuberculosis was found quite a large number of animals showed sensitization at an annual test, when using 3 mgm. per c.c. P.P.D. Onderstepoort tuberculin. When testing half strength against full strength tuberculin in this herd, the number of reactors with the half strength were statistically significantly smaller than those with the full strength tuberculin.

2. In testing full strength against half strength tuberculin on a positive herd of 62 animals, the results were:—

39 positives were found with 3 mgm. and 31 with 1·5 mgm.

10 retests were found with 3 mgm. and 16 with 1·5 mgm.

13 negatives were found with 3 mgm. and 15 with 1·5 mgm.

If one can make a statistical analysis of only 62 animals, then the difference in the number of positives (39 and 31) even though some of these are false positives, is not highly significant but nevertheless significant.

However, when one considers the total number of reactors (positives and retests) to full strength and half strength which are 49 and 47 respectively, the difference is statistically insignificant.

Since some of these so-called positives were believed to be actually free from the disease and others in a state of generalization, this herd was not considered to be very suitable for testing out the half strength against the full strength tuberculin.

3. In the case of 18 animals showing what is regarded as normal sensitization to tuberculin, full strength, half strength and 20 per cent full strength tuberculin gave for all practical purposes comparable end results. The size of the reacting swelling seems to be closely related to the potency of the tuberculin used.

4. Taking the above results into consideration, it would seem that a very much less potent tuberculin than is ordinarily being used, would be suitable for the single intradermal test, with the advantage that a smaller number of animals having a tuberculin sensitization to non-specific factors, will be condemned for tuberculosis, when they are in fact false positives.

5. Owing to the small numbers of animals dealt with, one is not justified in laying down what the optimum concentration of tuberculin, for the single intradermal test should be, but a case seems nevertheless to have been made out for a critical examination of the whole question, on a sufficiently large number of animals, using at the same time also sufficient animals, infected with conditions such as skin lesions, Johne's Disease, Actinomycosis etc., showing tuberculin non-specific sensitization.

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