THE VERNES TEST FOR THE DIAGNOSIS OF TUBERCULOSIS IN DAIRY CATTLE

AN INVESTIGATION ON ITS APPLICABILITY

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(With 1 Chart)

Introduction

In a series of publications issued from the Institut Prophylactique in Paris, Vernes and his co-workers (1926) described a flocculation method for the diagnosis of tuberculosis in man. This method was first elaborated for the diagnosis of syphilis. Various modifications were then introduced and it was found that the test could be applied to the diagnosis of human tuberculosis. Finally a standard technique was laid down. This consisted of measuring by means of the Vernes-Bricq-Yvon photometer the amount of flocculation which took place during 4 hours' incubation at 20° C. when 0·6 c.c. of resorcinol (1·25 per cent.) was added to an equal volume of the blood serum under test. Readings were taken immediately the resorcinol was added and again at the end of the incubation period. The difference between these readings gave the value on which the diagnosis was made. If this were below 30 the serum was considered normal. Values over 30 indicated infection, the higher the number the more advanced the infection. Correlation of these figures with clinical examination showed that very frequently the photometer value confirmed the clinical findings, and that in some cases the course of the disease could be forecast and followed by testing a number of blood samples over a period.

Boyer and Placidi (1931) reported that, using the identical technique described by Vernes for human tuberculosis, it had been possible to diagnose with a high degree of accuracy tuberculosis in bovine animals. Values of over 30 were recorded in 80 per cent. of the tuberculous sera tested, whilst in 77 per cent. of the normal sera the readings were less than 10.

The diagnosis of tuberculosis in cattle in this country depends almost entirely upon the tuberculin test in one or other of its forms. This test is known to give good results where the disease is well developed, but where the case is a border-line one the results are often misleading. The doubtful reactor is therefore a problem in tuberculin-tested herds, and any test which could give definite information in such cases would be extremely valuable. In view of the excellent results reported by Boyer and Placidi, it was decided to investigate the Vernes test in this respect.

The importance of testing only authentic sera was realised at the outset. Three groups were therefore set up and only sera which belonged strictly to one of these were used.

Group I or the normal group consisted of sera from non-reacting cows from tuberculin-tested herds, chiefly the N.I.R.D. herd.

Group II or the reactor group, sera from cows which had reacted recently to the tuberculin test.

Group III or the tubercular group, sera from cows which showed symptoms of advanced tuberculosis and typical lesions of active tuberculosis when examined post-mortem. The sera in this group were obtained by the courtesy of certain veterinary surgeons. The blood sample was taken immediately before the animal was slaughtered. If active tuberculosis was confirmed at post-mortem examination the serum was separated and submitted for testing together with a record of the clinical and post-mortem findings.

In each case the age of the cow, breed and stage of lactation were noted, and as far as possible heifers, dry cows and cows in milk were included in each group.

Method of taking samples. Approximately 50 c.c. of blood were withdrawn from the jugular vein into a sterile bottle. This was incubated at 37° C. for 6 hours and then the serum separated in the ice chest. Twenty-four hours after the sample had been taken, 5–10 c.c. of the serum were pipetted off and centrifuged for 10 min. at 4000 r.p.m. to remove any red cells. The sera with one exception (a tubercular serum which arrived turbid and failed to clear on centrifuging) were crystal clear and had, as was shown by the controls, photometer values which lay within surprisingly narrow limits. This in itself is interesting, since in ruminants whose digestive process is a more or less continuous one, a turbid fatty serum might be expected. Vernes particularly lays down in his technique for taking human blood samples that the subject should be in a fasted condition or the serum will be turbid and useless for photometric purposes. The colour of the sera varied from light straw to dark amber, but the tint had apparently no effect on the original photometer reading or the subsequent reaction.

In several cases sera were tested at intervals over a long period in order to see whether ageing had any effect on the flocculating properties. The conclusion reached was that, provided sera were prepared under aseptic conditions and kept in the ice chest free from contamination and centrifuged before testing, very little change occurred in the reaction with resorcinol even up to a week or 10 days.

Technique

The technique employed was exactly that described by Vernes and his collaborators (1926), namely, 0·6 c.c. of blood serum was mixed with an equal volume of 1·25 per cent. resorcinol and the mixture incubated at 20° C. for 4 hours. Readings were taken by the photometer immediately on adding the resorcinol and again at the end of the incubation period. The difference between these two readings gave the value by which the degree of the reaction was expressed. Particular attention had to be paid, however, to one or two points as they were found to be of importance in getting strictly comparable results.

Resorcinol. A large supply of resorcinol (British Drug Houses, Ltd., A.R.) was obtained at the beginning of the experiment and used throughout. A sample of the "Poulenc" resorcinol recommended by Vernes was obtained and a 1·25 per cent. solution tested out against

the B.D.H. resorcinol in similar concentration on duplicate portions of ten sera. The agreement in every case was found to be within the experimental error, *i.e.* 3 units.

Solutions of different strengths were made up in glass-distilled water, and stored in amber bottles. Fresh solutions were prepared frequently since they were found to become brown if stored too long, although this did not seem to affect the serum reaction in any way.

Measurement of serum and reagents. Originally the rheometer recommended by Vernes was used, but later, when the technique was being modified, it was found more convenient and accurate to use graduated 1 c.c. pipettes. The rheometer is undoubtedly an excellent piece of apparatus for routine work where large numbers of samples have to be dealt with in as short a time as possible, but its use is limited where greater accuracy is required.

Care of the photometer cells. Rinsing with distilled water after use is reckoned by Vernes to be sufficient to keep the cells clear and in good condition. In the hands of the present workers however, this was not so, since cells so treated rapidly became coated on their inner surfaces with an opaque film of the serum/resorcinol mixture. If allowed to accumulate, this affected the readings quite considerably and certainly reduced the accuracy of the technique. Good results were obtained by rinsing immediately after use in running water and washing the inside of the cell gently with a small pad of chamois leather dipped in soapy water. This was followed by a thorough rinsing in running water and distilled water and the cells were dried by inverting on filter paper under a small bell-jar. By this means they were always crystal clear when used, and readings taken of distilled water blanks from day to day showed practically no variation.

Adjustment of the photometer. Before use, the standardisation of the photometer was always checked. The apparatus is very finely constructed and on no occasion during this experiment was any more than the slightest adjustment found to be necessary. It is a highly sensitive piece of apparatus and has given accurate and reproducible results throughout.

Control of the source of light. A system of wet cells with a total voltage of 8 was used. The current was led through a rheostat and stepped down to 6 volts. The current passing through the lamp was therefore kept constant at 6 volts.

Accuracy of reading. Each serum sample was tested in duplicate with controls and each value was checked by two independent people reading in duplicate. A serum therefore received a total of eight readings both before and after incubation. Mean values were taken in order to arrive at the final value. The same two people were responsible for reading throughout the experiment, and it was found that, with practice, an accuracy well within the 3 units laid down by Vernes as experimental error was possible.

Incubation. Variations in temperature, as stated by Vernes, were found to make a considerable difference to the rate of flocculation, and in order to control the temperature conditions of the laboratory certain precautions were necessary. Hence immediately the original reading had been taken the serum/resorcinol mixture was returned to the tube and the tube closed with a rubber stopper and placed in a water bath at 20° C. When all the readings of the series had been taken, the tubes were removed from the water bath, placed in racks in an incubator carefully regulated to 20° C. and incubated for 4 hours.

RESULTS

A total of ninety-four sera were tested exactly according to the technique laid down by Vernes, *i.e.* 0·6 c.c. serum : 0·6 c.c. 1·25 per cent. resorcinol, the initial reading being taken at the time of mixing, the final after incubating at 20° C. for 4 hours. The distribution of these sera between the three groups referred to above and the values obtained are shown in Table I.

Two things emerge from a study of these figures: (1) that the technique as it stands does not distinguish between the three types of serum, and (2) that in

none of the cows of the tubercular group was the value greater than 44. When it is remembered that the post-mortem findings reported for these cows were "advanced generalised miliary tuberculosis," the values obtained are, according to Vernes' standards, out of all proportion to the extent of the disease. Boyer and Placidi (1931) state in their paper that using this technique they obtained readings of 30 and over in 80 per cent. of tuberculous animals, and readings of 10 and under, in 77 per cent. of normal animals. Further they reported the highest values where the infection was in an active miliary state and quoted readings of 108, 124 and 128 as typical. It is difficult to reconcile their results with the present findings, the more particularly as the latter support a statement of Ralph and Davies (1930) to the effect that the test gave no results of diagnostic value with bovine sera. They found, in fact, that the optical indices obtained from infected animals were sometimes actually lower than those of normal animals, an observation which has been repeatedly made in the present work. Personal communications have been received from other workers and the consensus of opinion appears to be that the test has a certain value in human tuberculosis but that it is inapplicable in its original form to the diagnosis of tuberculosis in cattle.

Table I

Type of serum	No. of samples	Photometric values			
		Highest	Lowest	Mean	Median
Normal	54	43	4	17	15
Reactor	22	32	5	15	11
Tubercular	18	44	3	14	13

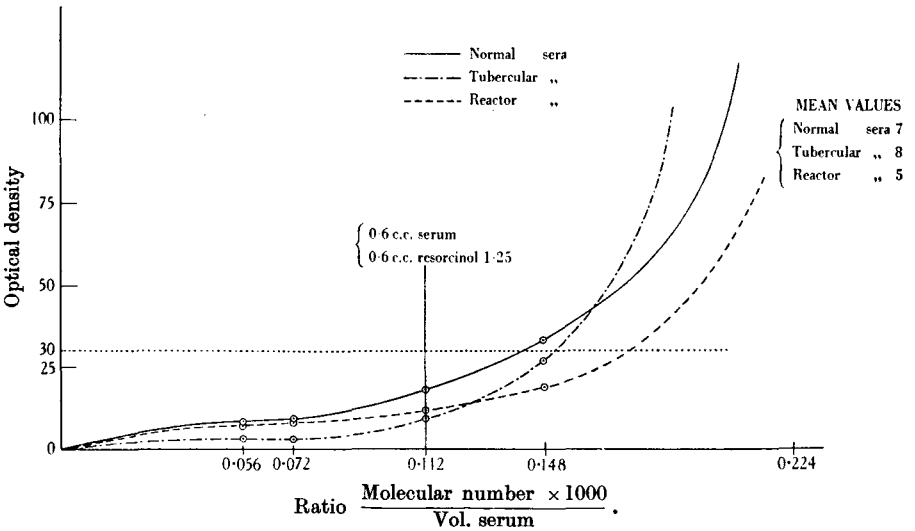
The foregoing results were so unsatisfactory that it was decided not to waste time amplifying the number of tests, but rather to concentrate on modifying the technique to see whether in some form or other it could be made to differentiate between normal and tubercular bovine sera.

In devising his standard, Vernes plotted the values obtained for normal and tubercular sera when increasing proportions of resorcinol were added to a constant volume (0·6 c.c.) of serum. These values were plotted to a special scale and on studying the curves it was found that those of tubercular sera lay well above normal and were much steeper, *i.e.* flocculation in tubercular sera was more rapid and intense than in the normals. The point at which the difference between the two types of curve was greatest was taken as the standard of differentiation, in this case, equal proportions of serum/resorcinol 1·25 per cent. From this it was laid down that a value of 30 and under could be taken, as a general rule, to indicate a normal serum, whilst 30 and over was evidence of tubercular infection.

This work was repeated exactly, using in place of human sera, normal, reactor and tubercular bovine sera. These three groups contained seven, five and eight samples respectively. The values obtained in each group were so close to one another that instead of plotting each sample individually the mean values were determined and used. Table II shows the mean and median values

and the chart the curves for the three groups. These are plotted to the scale used by Vernes' in his original work (p. 33).

It is clear at once that these curves differ essentially from Vernes. In the first place they follow one another very closely and there is no point at which the tubercular can be distinguished from the other two types of serum. Secondly, they confirm the results of the ninety-four sera previously recorded, since at ratio 0·112, which is Vernes' standard, the mean values lie between 10 and 20. The highest individual value in the tubercular group was 44. This further strengthens the conclusion that human and bovine tubercular sera react very differently to flocculation with resorcinol, and that the conditions under which infected and non-infected sera can be distinguished in human tuberculosis do not apply to bovine sera.



Before issuing any report on the foregoing findings, it was decided to modify the technique in various ways to see whether a combination of factors could be found which would succeed in differentiating between normal and tubercular sera. The modifications tried and the results obtained are shown below.

Table II

		Ratio				
Type of serum		0·056	0·072	0·112	0·148	0·224
Normal	Mean	8	9	18	33	∞
	Median	4	8	21	35	∞
Reactor	Mean	3	4	7	23	∞
	Median	3	3	9	27	∞
Tubercular	Mean	11	13	18	26	∞
	Median	7	8	13	19	∞

The proportion of serum to resorcinol. Kfouri (1931) reported the application of the test to tuberculosis in horses and obtained good results using 0·8 c.c. serum : 0·4 c.c. resorcinol 3·5 per cent. His technique was used in this in-

vestigation for a total of twelve bovine sera, five normal, four reactor and three tubercular. The results are shown in Table III.

Table III

Type of serum	No. of samples	Photometric values			
		Highest	Lowest	Mean	Median
Normal	5	89	28	58	64
Reactor	4	28	2	11	10
Tubercular	3	18	2	8	—

The numbers of sera are very small, but it is clear even from these that Kfouri's results do not apply to bovine sera. It seems in fact, as though the reverse tended to hold, *i.e.* that the values of the normals were higher than the reactors or the tuberculars, but even this is not constant, and the values from serum to serum are too erratic to be of diagnostic value.

Keeping the proportions constant at 0·8 : 0·4, the concentration of resorcinol was then varied. Thirteen sera, seven normals and six reactors, were tested with concentrations varying from 1·5 to 4 per cent. The mean and median values obtained are shown in Table IV. Known tubercular sera were not available at the time.

Table IV

Serum		Concentration of resorcinol %					
		1·5	2	2·5	3	3·5	4
Normal (7 samples)	Mean	5	3	6	13	58	∞
	Median	6	3	6	11	64	∞
Reactor (6 samples)	Mean	3	3	2	3	11	∞
	Median	4	3	2	3	10	∞

0·7 *c.c. serum :* 0·5 *c.c. resorcinol.* Three normal sera were tested with concentrations of resorcinol from 1·25 to 2 per cent.

Serum	Concentration of resorcinol %			
	1·25	1·5	1·75	2
Normal 1	6	7	10	11
2	7	7	12	14
3	7	5	9	11

1 *c.c. serum :* 0·2 *c.c. resorcinol.* Only one reactor serum was tested. Concentrations of resorcinol 2·5–10 per cent.

Serum	Concentration of resorcinol %			
	2·5	5	7·5	10
Reactor	4	6	0	6

0·4 *c.c. serum :* 0·8 *c.c. resorcinol.* One reactor serum was tested with concentrations of resorcinol 1·5–3 per cent.

Serum	Concentration of resorcinol %			
	1·5	2	2·5	3
Reactor	∞	∞	∞	∞

Time and temperature of incubation. One normal and three tubercular sera were treated with 1·25 per cent. resorcinol in the ordinary way and duplicate

portions incubated at 20, 30, and 40° C. Readings were taken after 4 hours. The values obtained are shown in Table V.

Table V

	Temperature of incubation °C.		
Serum	20	30	40
Normal	0	4	20
Tubercular 1	2	1	2
2	6	5	1
3	13	34	42

This shows that the higher temperature increased the values of the normal and the third tubercular serum, but this did not apply to the other two sera where the reaction was apparently unaffected. Prolonged incubation up to 24 hours was found to be ineffective in that changes occurred in the controls at all three temperatures, and rendered the serum/resorcinol values invalid. The reaction is clearly sensitive to temperature conditions up to a point and in view of this great care has always been taken to ensure that the standard test has been carried out exactly at 20° C.

Other precipitants. The relative values of resorcinol, phenol and phloroglucinol as precipitant were tested. 1·25 per cent. solutions were prepared and added to three sera, one normal and two tubercular. The results are shown in Table VI.

Table VI

	Precipitant		
Serum	Resorcinol	Phenol	Phloroglucinol
Normal	14	15	37
Tubercular 1	11	5	91
2	4	9	14

Resorcinol and phenol appear to have about the same powers of flocculating serum whilst phloroglucinol is considerably in excess of either. None of them, however, appears to distinguish between normal and tubercular sera as is reported and shown in a series of curves by Vernes for human sera (pp. 38 and 39).

Inactivation of serum. A typical normal and a typical tubercular serum were used. Two portions of 5 c.c. were taken of each, and one was tested without further treatment, the other after heating in a water bath at 56° C. for 30 min. Varying concentrations of resorcinol were used. The results are shown in Table VII.

Table VII

	Concentration of resorcinol %			
Serum	1·25	2·5	4	5
Normal (untreated)	7	33	∞	∞
(inactivated)	5	7	6	∞
Tubercular (untreated)	10	36	∞	∞
(inactivated)	0	1	5	∞

Inactivation of the serum has a decided effect on the reaction to resorcinol. It appears that some precipitable constituent is destroyed or reduced in quantity, since the readings are distinctly lower as compared with the untreated

duplicates. The substance or substances concerned are unfortunately destroyed to an equal extent in the normal and the tubercular serum, hence no differentiation between the two is apparent.

Treatment of the serum with CO_2. When blood is taken from an animal certain changes occur in the sample on storage. Chief among these is the loss of CO_2 with resultant increase in *p*H. It was thought that this might have some effect on the flocculation with resorcinol and that by saturating with CO_2 at 0° C. by the method described by Gray (1933), the CO_2 conditions could be made to approach those of freshly drawn blood, in which condition it might be possible to distinguish between normal and infected sera by the test. One normal and one tubercular serum were used. 20 c.c. were taken and centrifuged free from red cells. 10 c.c. were then removed and placed in a special boiling tube through which a current of CO_2 was passed at 0° C. for 5 min. The tube was then corked and shaken and allowed to stand at 0° C. for 18 hours. The treated and untreated portions were tested in the ordinary way. Care was taken during the test to prevent the loss of CO_2 from the treated serum. The results are shown in Table VIII.

Table VIII

	Optical density	
Serum	Untreated	Treated CO_2
Normal	8	37
Tubercular	8	32

The effect of the CO_2 treatment seems to be to increase the values of both types of serum but again there is no differentiation. When these sera were then inactivated by heating at 56° C. for 30 min. and retested, the values showed a considerable drop, the normal untreated to 1, treated to 4, the tubercular untreated to 0, treated to 2. This was in agreement with the findings previously recorded for inactivated sera.

Addition of tubercle protein to a normal serum. A small quantity of tubercle protein was added to 5 c.c. of a normal serum, well mixed, and allowed to stand for 5 min. at room temperature. The undissolved portion was centrifuged off and the supernatant serum tested in the ordinary way with duplicate portions of the untreated serum as control. The mean values obtained were 7 in the case of the treated serum and 10 in the control, showing that tubercle protein as such does not seem to be the agent responsible for increased flocculation with resorcinol.

Conclusions

The numbers of sera tested by the various modifications are admittedly small and no hard and fast conclusions can be drawn. They do show, however, that under the conditions used there is no gross difference between the reactions of normal and tubercular sera. These conditions have, of course, been by no means exhausted, but an immense piece of work will have to be undertaken

if the position is to be investigated further. The test is attractive on account of its speed and simplicity and would be, if it could be applied, a valuable means of confirming the tuberculin test. It presents certain fundamental difficulties in the case of bovine sera and it may be that the variations in composition of the blood serum of a single cow or from one cow to another are too great to allow of any clear distinction being made between a normal and an infected animal by such empirical means as are implied in this test. The purpose of the present report is not to suggest a working alternative but rather to show that, contrary to the findings of Boyer and Placidi, the test as it stands does not differentiate between normal bovine animals and those infected with tuberculosis.

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