THE REDUCTASE TEST OF BARTHEL AND JENSEN.

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The reducing power of milk for methylene blue was first demonstrated by Neisser and Wechsburg (1900). Later, Schardinger (1902) showed that by the addition of formaldehyde to fresh milk methylene blue was also reduced. The first reaction is generally conceded to be due to the bacteria and leucocytes present in the milk, the second reaction, the indirect reduction test, to an aldehyde reductase which is now associated with the fat globules.

Barthel (1907) and Jensen (1909) made use of the direct reductase test as a means of estimating the approximate number of bacteria per c.c. in milk. A considerable amount of work has been done on the continent in regard to this test, but in this country it has not been utilised much. Cunningham and Thorpe (1921) examined 40 samples of milk. They compared the reductase test with the counts obtained by the dilution method on whey agar after incubating for ten days at 22° C. They found that in general the reductase test compared well with bacterial counts and was a useful test when a rough estimate was required quickly.

Accordingly it seemed desirable to make an extended comparison of the bacterial content of milk samples as estimated by the dilution method and by the reductase test.

Technique.

Bacterial counts. Melted nutrient agar tubes were inoculated with 1 c.c. of the various dilutions. Plates were then poured and incubated at 38° C. for 48 hours.

Reductase test. The method of Jensen (1921) was closely followed. A solution of methylene blue was made, one tablet (obtained from Blauenfeldt and Tvede of Copenhagen) being dissolved in 200 c.c. of sterile water. The milk samples to be tested were well shaken and 40 c.c. of each sample were pipetted into tubes 2 cms. by 20 cms. One cubic centimetre of the methylene blue solution was added. The tubes were then corked and the methylene blue was equally distributed by vigorous shaking. The tubes were then placed in a water bath at 38° C.

Jensen gives the following time limits as indicating the approximate number of bacteria per c.c.:

1. If the milk is not decolourised in five and a half hours there are less than half a million organisms per c.c.

2. If the milk is decolourised in less than five and a half hours, but not less than two hours, then the milk contains a half to four million per c.c.

3. If the milk is decolourised in less than two hours but not less than 20 minutes, the milk contains four to 20 million per c.c.

4. If the milk is decolourised in less than 20 minutes then there are more than 20 million organisms per c.c.

Discussion of Results.

In all 132 milk samples have been so tested and it has been found that comparable results have been obtained in 83 per cent. of the samples. In 17 per cent., or 23 samples, discordant results were obtained.

Fifty-nine samples of milk gave a bacterial count of under 100,000 per c.c. The reductase test indicated that all these were under the half-million standard.

Thirty-six samples gave bacterial counts from 100,000 to 500,000. In 13 of these samples the reductase test indicated a half to four million organisms.

Twenty-six samples gave bacterial counts of a half to four million organisms. The reductase test showed a discrepancy in seven samples. It indicated that five samples contained from four to 20 million per c.c. One sample contained more than 20 million and one sample contained less than half a million organisms per c.c.

Nine samples gave counts between four million and 20 million. The reductase test indicated over 20 million in three of these.

Two samples showed a count of over 20 million and the reductase test confirmed this.

The following table shows the bacterial count as compared with the estimate given by the reductase test in those samples showing a discrepancy:

No.	Plate counts, per c.c.	Reductase test, millions per c.c.	No.	Plate counts, per c.c.	Reductase test, millions per c.c.
1	156,000	½ to 4	13	150,000	½ to 4
2	480,000	½ „ 4	14	2,400,000	Over 20
3	100,000	½ „ 4	15	1,020,000	4 to 20
4	320,000	½ „ 4	16	700,000	4 „ 20
5	230,000	½ „ 4	17	800,000	4 „ 20
6	400,000	½ „ 4	18	900,000	Under ½
7	300,000	½ „ 4	19	3,000,000	4 to 20
8	140,000	½ „ 4	20	3,640,000	4 „ 20
9	100,000	½ „ 4	21	12,000,000	Over 20
10	420,000	4 „ 20	22	8,200,000	„ 20
11	430,000	½ „ 4	23	5,000,000	„ 20
12	140,000	½ „ 4			

Conclusions.

It has been found that the reductase test gives a considerably higher estimate of the number of organisms present in some milk samples than the plate method. This, however, is doubtless due to the fact that many colonies obtained by plating do not arise from a single cell but from chains of cocci and bacilli. Again the plate method takes no account of the anaerobic bacteria present in the milk.

When there are more than 100,000 organisms per cubic centimetre in the milk it would seem that the reductase test gives just as accurate an estimate as the plate method.

In its present form, however, the test would not be of service in grading of milk according to the bacterial standards now suggested.

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