THE USE OF THE FERMENTATION-REDUCTASE TEST FOR THE GRADING OF MILK.

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PART I.

Enquiries have frequently been received at the National Institute for Research in Dairying concerning the efficiency of the Fermentation-Reductase Test as a method for the grading of milk, and the suggestion has been made that this test, which is now in use in some European countries, might with advantage be substituted for the standard methods of counting bacteria and demonstrating the presence or absence of *B. coli*, which at present form the basis for the grading of milk in England. A study of this question was therefore undertaken, and since similar enquiries are still being received, it is thought advisable to publish a résumé of the results that have already been obtained.

The Fermentation-Reductase Test has its extreme simplicity to recommend it. Elaborate apparatus and trained workers are not essential. The test is based on the fact that bacteria have the power of reducing various dyes, amongst them methylene blue. If, therefore, methylene blue is added to milk, the bacteria present in the milk react with the dye and the blue colour eventually disappears. The greater the number of bacteria present the more rapid should be the loss of colour. Theoretically, then, the rate of reduction of the methylene blue should be a measure of the number of bacteria present in the milk. The test has been worked out by Barthel and Orla-Jensen (1912), and the following scale has been drawn up by them and is now adopted in various European countries as a basis of payment for milk supplies.

Reductase Test.

To 40 c.c. of milk 1 c.c. of a standard solution of methylene blue is added. The contents of the tube are carefully mixed and the tube is placed in a water bath maintained at a temperature of 38° to 40° C. The length of time which elapses before the blue colour disappears is noted. The samples of milk are then classified as shown in Table I.

Fermentation Test.

The tubes of milk are allowed to remain in the water bath at 38°–40° C. for 24 hours, when the type of curd is noted. Four types are recognised: (1) "Gelatinous," a normal lactic acid curd, (2) "Blown," indicating the presence of gas-producing organisms, (3) "Spongy," which differs from the "blown" curd in being finer in texture, (4) "Cheesy," in which there is a marked

separation of clear whey indicating the presence of organisms producing rennet-like enzymes (Orla-Jensen, 1921, p. 164). Of these, the "blown" type and extreme cases of "sponginess" are considered objectionable, and consequently their presence reduces the milk to a lower class even though the reduction time is within the prescribed limits. In actual practice this degrading is made effective only for those samples which are, in the first instance, placed in Classes II or III. It is not usually considered necessary to observe the Fermentation Test for those samples which are placed in Class I.

Table I.

Class	*Reduction time.* Length of time which elapses before the blue colour disappears	Bacterial count per 1 c.c. assumed to be approximately
I (Good milk)	5½ hours (or more)	500,000 (or less)
II (Fair average quality)	2–5½ hours	From 500,000 to 4,000,000
III (Bad milk)	20 mins.–2 hours	From 4,000,000 to 20,000,000
IV (Very bad milk)	20 mins. (or less)	20,000,000 (or more)

Comparison between the two Systems of Grading of Milk.

In the course of this study numerous samples of milk have been examined in the laboratory; these included Certified, Grade "A" and ordinary Commercial milk. A comparison has been made between the bacterial count as determined by plating on standard agar, and the reduction time as determined by the Reductase Test carried out according to the instructions given (Orla-Jensen, 1921, p. 169); also the evidence of the presence of coliform organisms as indicated by inoculation into bile salt lactose broth has been compared with the occurrence of gassy curd in the Fermentation Test.

(*a*) *Reductase Test and Plate Counts.*

In Table II a comparison has been made between the two systems of grading. During the course of the work it was found that no samples of milk of Grade "A" or Certified standard had a reduction time of less than 5½ hours; therefore, in this Table, Class I on Orla-Jensen's scale has alone been included and examined in closer detail. This class contained 166 samples of milk, and on this system of grading each of these would be termed "good milk." These samples have been graded on the English system also, according to their bacterial counts, that is, those samples which had counts not exceeding 30,000 per c.c. have been classified as of Certified standard; those samples having counts greater than 30,000, but not exceeding 200,000 per c.c. have been classed as of Grade "A" standard; and those having bacterial counts exceeding 200,000 per c.c. have been classed as of ordinary "Commercial" standard.

A study of Table II clearly shows that the two systems of grading are not

interchangeable. Of 166 samples placed in Class I on Orla-Jensen's scale, only 106 were of Certified standard, 33 were of Grade "A" standard, whilst 27 were below the Grade "A" standard, in 10 instances having bacterial counts running into millions. Orla-Jensen's scale, therefore, does not satisfy the requirements of the English system of grading, since it does not discriminate between milk of an average quality and milk of a superfine quality.

Table II.

	Type of Milk	Age of samples	English system of grading: Number of samples of Certified standard. Bacterial count not exceeding 30,000 per c.c.	English system of grading: Number of samples of Grade "A" standard. Bacterial count exceeding 30,000 per c.c. but not exceeding 200,000 per c.c.	English system of grading: Number of samples of Commercial standard. Bacterial count exceeding 200,000 per c.c.	Orla-Jensen's scale. Number of samples in Class I. Reduction time 5½ hours (or more)
		hours				
I	Certified	23–24	13	1	0	14
II	„	23–24	9	0	0	9
III	„	23–24	8	0	0	8
IV	„	33–35	13	0	0	13
V	Grade "A"	23–24	23	1	0	24
VI	„	19–20	10	0	0	10
VII	„	14–16	16	2	0	18
VIII	Commercial	14–16	12	4	2	18
IX A	„	19–20	1	18	11	30
IX B	„	19–20	0	0	4	4
IX C	„	5–6	0	1	2	3
X	„	19–20	0	4	5	9
XI	Surplus	Unknown	1	2	3	6
	Totals	—	106	33	27	166

It is not usually claimed for the Reductase Test that it provides an exact method for the estimation of the number of bacteria in milk. It is claimed, however, that samples of milk which are placed in Class I on Orla-Jensen's scale have, as a rule, a bacterial content of less than 500,000 per c.c., a figure which has been arrived at by a comparison of this method with the results obtained by means of plate counts. The standard set for milk of Class I is thus seen to be lower than that set for Grade "A" or Certified milk in this country. The test cannot, therefore, be expected to provide a satisfactory means of discriminating between Commercial, Grade "A" and Certified milk unless it can be modified to meet the requirements of this system of grading. The question as to how far it is true that samples of milk having a reduction time of 5½ hours or more have bacterial counts of less than 500,000 per c.c. will be discussed in Part II.

(*b*) *Comparison of Fermentation Test and Presumptive Coliform Test.*

In both systems of grading importance is attached to the demonstration of the presence or absence of *B. coli* in the milk. The production of gas in the

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tubes of bile salt lactose broth (English system) and the occurrence of a "blown" curd in the Fermentation Test are regarded as indications of imperfect methods of handling the milk.

Table III gives a comparison between the presence of coliform organisms as shown by inoculation of the milk into bile salt lactose broth, and the occurrence of "gassy" curd in the Fermentation Test. All cases of gassy curd have been included, whether of the "blown" or "spongy" types. The results demonstrate that the Fermentation Test does not afford a reliable indication of the presence of coliform organisms. This seems to be particularly true of some types of milk, *e.g.* IX A, *Commercial*, in which, of 30 samples tested by

Table III.

	Type of milk	Age of sample hours	Number of samples	Presumptive coliform test: Number of samples in which *B. coli* demonstrated in 1 c.c.	1/10 c.c.	1/100 c.c.	1/1000 c.c.	Total	Fermentation test: Number of samples showing "Blown" curd	"Spongy" curd	Total
I	Certified	23–24	14	0	—	—	—	**0**	0	0	**0**
II	„	23–24	9	0	—	—	—	**0**	0	0	**0**
III	„	23–24	9	0	—	—	—	**0**	0	1	**1**
IV	„	33–35	14	4	—	—	—	**4**	0	0	**0**
V	Grade "A"	23–24	24	1	—	—	—	**1**	1	0	**1**
VI	„	19–20	10	2	—	—	—	**2**	0	0	**0**
VII	„	14–16	18	2	—	1	—	**3**	1	0	**1**
VIII	Commercial	14–16	18	1	4	3	—	**8**	1	0	**1**
IX A	„	19–20	30	—	—	5	25	**30**	0	4	**4**
IX B	„	19–20	4	—	—	—	4	**4**	2	0	**2**
IX C	„	5–6	3	—	—	—	3	**3**	2	0	**2**
X	„	19–20	16	—	1	6	9	**16**	8	1	**9**
XI	Surplus	Unknown	21	—	2	4	15	**21**	15	1	**16**
	Totals	—	190	10	7	19	56	**92**	30	7	**37**

inoculation into bile salt lactose broth, each showed the presence of coliform organisms, in 25 cases even in a dilution 1/1000 c.c., while in no case was a "blown" curd obtained in the Fermentation Test, and on only four occasions was the curd slightly "spongy." Of 190 samples tested, 92 showed the presence of coliform organisms, in 56 cases even in a dilution of 1/1000 c.c., but in 37 instances only was any gas detected in the curd by means of the Fermentation Test.

CONCLUSIONS.

(1) The Fermentation-Reductase Test does not, in its present form, constitute a reliable method for discriminating between Certified, Grade "A" and Commercial milk. The first two grades and frequently even poor samples of the last may all be placed together in Class I on Orla-Jensen's scale.

(2) The Fermentation Test does not provide a delicate test for the presence of coliform organisms in milk.

N.B. The terms "Certified" and "Grade 'A'" milk denote milk which was sold under Certified or Grade "A" licenses. The terms "milk of Certified

standard," and "milk of Grade 'A' standard" are intended to denote any milk, licensed or otherwise, the bacterial count of which was within the limits set for these grades.

PART II.

In Part I a summary has been given of the results of a comparison between the grading of milk by means of the bacterial count and the presence or absence of coliform organisms, and by means of Orla-Jensen's Fermentation-Reductase Test. Attention was drawn to the fact that the latter test does not satisfactorily discriminate between milk of Certified, Grade "A" and ordinary Commercial standards. Frequently milk with a bacterial content of millions was included in Class I together with milk of Certified and Grade "A" standards.

The system of grading according to the Reductase Test may be considered from two points of view. Samples of milk which are placed in Class I should have reduction times of $5\frac{1}{2}$ hours or more, but they should also have bacterial counts of less than 500,000 per c.c. The other classes may be considered similarly (cf. Table I). In Part II it is proposed to examine whether or not such agreement is attained in practice.

Table IV gives the bacterial count and corresponding reduction time for each sample of milk having a bacterial count of more than 500,000 per c.c. Such samples should have a reduction time of less than $5\frac{1}{2}$ hours, and should therefore be placed in the lower classes of Orla-Jensen's scale.

Table V shows the bacterial count obtained for all samples the reduction times of which were between 5 and 10 hours, *i.e.* which lay in the neighbourhood of the minimum reduction time for milk of Class I on Orla-Jensen's scale.

Table IV shows that 35 of the samples of milk which were examined gave bacterial counts in excess of 500,000 per c.c. Of these, 13 are correctly placed on Orla-Jensen's scale with respect to both bacterial count and reduction time. The bacteriological classification of the remainder (22 samples) does not agree with that of Orla-Jensen's scale. When the samples having bacterial counts ranging from 500,000 to 10,000,000 per c.c. are considered, it is found that 10 of them had a reduction time of $5\frac{1}{2}$ hours or more, and thus would be placed in Class I on Orla-Jensen's scale, although their bacterial counts were in some cases far in excess of that regarded as the approximate maximum for this class. Of the remaining 17 samples with bacterial counts exceeding 10,000,000 per c.c., 3 had reduction times of $5\frac{1}{2}$ hours or more, *i.e.* would be placed in Class I on Orla-Jensen's scale. Two of the samples considered in the Table were Certified milk and were correctly placed on the scale; the remainder were samples of Commercial milk which were being sold for domestic purposes or for cheese-making.

From this table it would appear that if this system of grading were applied to such samples of milk as have been examined, the reduction time of $5\frac{1}{2}$ hours adopted as the minimum for Class I would be too low to insure the exclusion

Table IV.

	Type of milk	Age of sample	Bacterial count per 1 c.c.	Reduction time	Class on Orla-Jensen's scale
		hours		hours	
1	Commercial	19–20	540,000	$5\frac{1}{4}$	II*
2	,,	19–20	*605,000*	*12*$\frac{1}{4}$	*I*
3	,,	19–20	*629,000*	*10*$\frac{1}{4}$	*I*
4	,,	19–20	*758,000*	*7–9*$\frac{1}{4}$	*I*
5	,,	19–20	1,000,000	2	II*
6	,,	19–20	1,130,000	$4\frac{1}{4}$	II*
7	Certified	33	1,200,000	4	II*
8	Surplus	Unknown	1,410,000	$4\frac{1}{2}$	II*
9	Commercial	19–20	*1,490,000*	*10*$\frac{3}{4}$	*I*
10	,,	19–20	*1,800,000*	*6–8*$\frac{1}{4}$	*I*
11	Certified	23–24	2,000,000	2–4	II*
12	Commercial	19–20	*2,400,000*	*8*	*I*
13	Surplus	Unknown	3,120,000	$4\frac{3}{4}$	II*
14	Commercial	19–20	3,280,000	$3\frac{1}{2}$	II*
15	,,	19–20	*4,600,000*	*9*$\frac{3}{4}$	*I*
16	,,	19–20	*5,320,000*	*10*$\frac{3}{4}$	*I*
17	,,	19–20	*5,670,000*	*5*$\frac{1}{2}$	*I*
18	,,	19–20	*10,000,000*	*6*$\frac{1}{2}$	*I*
19	,,	19–20	13,840,000	$4\frac{1}{2}$	II
20	,,	19–20	*14,200,000*	*9*$\frac{1}{4}$	*I*
21	,,	19–20	*17,600,000*	*6*$\frac{1}{4}$	*I*
22	,,	19–20	19,760,000	1	III*
23	,,	19–20	26,520,000	$2\frac{3}{4}$	II
24	Surplus	Unknown	27,300,000	2	II
25	,,	,,	27,300,000	$\frac{1}{2}$	III
26	,,	,,	28,900,000	$2\frac{1}{4}$	II
27	,,	,,	181,000,000	$<\frac{1}{4}$	IV*
28	,,	,,	272,000,000	$2\frac{3}{4}$	II
29	,,	,,	320,000,000	$\frac{1}{2}$	III
30	,,	,,	*430,000,000*	*5*$\frac{1}{2}$	*I*
31	,,	,,	900,000,000	$<\frac{1}{4}$	IV*
32	,,	,,	1,000,000,000	1	III
33	,,	,,	1,000,000,000	$\frac{1}{2}$	III
34	,,	,,	2,240,000,000	$<\frac{1}{4}$	IV*
35	,,	,,	3,000,000,000	$<\frac{1}{4}$	IV*

* Bacterial count and Reduction time in agreement with Orla-Jensen's scale.

of milk of high bacterial content from this class. Even if this time limit were extended, *e.g.* to 7 hours, there would still be a danger that milk having a bacterial count of more than 500,000 per c.c. might be placed in Class I.

A study of Table V demonstrates that samples of milk having the same reduction time often show a wide variation in bacterial content. For example, in the group having a reduction time of $5\frac{1}{2}$ hours, there is a sample of Grade "A" milk with a bacterial count of 2880 per c.c. But this group also contains 2 other samples of milk (Commercial) having bacterial counts of 5,670,000 and 430,000,000 per c.c. respectively. The next group (reduction time $6\frac{1}{4}$ to $6\frac{1}{2}$ hours) contains 3 samples with counts of 930, 8600, and 3000 per c.c. respectively, and also 2 samples having counts of 17,600,000 and 10,000,000 per c.c. respectively. For reasons not yet clear it sometimes happens that Grade "A" or Certified milk having a low bacterial count has a reduction time similar to that of milk of a poorer quality having a very high bacterial count. If, therefore, the time limit of $5\frac{1}{2}$ hours were extended as suggested above in order to exclude inferior samples of milk from Class I these samples of good milk would

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also be excluded. This question will be discussed further in Part III of this paper.

Table V.

Type of milk	Age of sample	Bacterial count per 1 c.c.	Reduction time	Class on Orla-Jensen's scale
	hours		hours	
Surplus	Unknown	90,000	5¼	II
Commercial	19–20	540,000	5¼	II
Grade "A"	23–24	2,880	5½	I
Commercial	19–20	5,670,000	5½	I
Surplus	Unknown	430,000,000	5½	I
Certified	23–24	930	6¼	I
,,	23–24	8,600	6¼	I
Grade "A"	23–24	3,000	6¼	I
Commercial	19–20	17,600,000	6¼	I
,,	19–20	10,000,000	6½	I
Grade "A"	14–16	48,600	8¼	I
Commercial	19–20	2,400,000	8	I
,,	19–20	1,800,000	6–8¼	I
,,	19–20	219,000	8¾	I
Good Commercial	14–16	37,400	9	I
,, ,,	14–16	758,000	7–9¼	I
Commercial	19–20	14,200,000	9¼	I
,,	19–20	4,600,000	9¾	I
,,	5–6	342,000	9¾	I
,,	19–20	78,000	9½	I
Surplus	Unknown	240,000	9¼	I
,,	,,	87,000	9½	I
,,	,,	342,000	9¾	I

Conclusions.

(1) If a reduction time of 5½ hours is retained as the minimum for milk of Class I on Orla-Jensen's scale, there is no certainty that milk of a very poor quality may not be included in this class.

(2) If the reduction time of 5½ hours is extended, there is a danger that milk of a very good quality may be excluded from Class I.

PART III.

It has already been shown that the Reductase Test in its present form does not differentiate between Certified, Grade "A" and ordinary Commercial milk. Since, however, the bacterial count of 500,000 per c.c., which is regarded as the approximate limit for milk of Class I on Orla-Jensen's scale, is greater than the limit set in this country for Grade "A" (200,000 per c.c.) or Certified (30,000 per c.c.) milk, it seemed useful to examine the possibility of establishing a satisfactory system of grading by raising the limit of reduction time from 5½ hours to higher values. It was shown in Part II that if the limit of 5½ hours were extended, there was a danger that samples of milk with a low bacterial content would be excluded from this class. If, however, it could be found that the error so caused was negligible in comparison with the advantages to be derived from an extended use of this test, the test might prove to be of great value to the milk producer and distributor.

With this aim in view the results of tests on 171 samples of milk from various sources have been examined. These samples included Certified milk from three farms, Grade "A" milk from three farms, Commercial milk from three farms, and "surplus milk" delivered at a dairy during hot weather to be made into cheese. One difficulty here encountered was the fact that very few samples could really be placed between the limits of the Grade "A" and Certified standards. In almost every instance the milk that was being sold as Grade "A" milk was, bacteriologically, up to Certified standard. The bacterial count of several of the samples of Commercial milk was of Grade "A" standard, but frequently these samples contained an excessive number of *B. coli*.

In Tables VI, VII, VIII, the samples have been graded according to their bacterial count only, without reference to the presence or absence of *B. coli*, *i.e.* samples with bacterial counts not exceeding 30,000 per c.c. have been classed as Certified, samples with bacterial counts greater than 30,000 per c.c. but not exceeding 200,000 per c.c. have been classed as Grade "A," samples with bacterial counts exceeding 200,000 per c.c. have been classed as Commercial.

Table VI.

Reduction time to be regarded as minimum for the class	Number of samples of milk included in the class: Certified standard. Bacterial count not exceeding 30,000 per c.c.	Number of samples of milk included in the class: Grade "A" and Commercial standards. Bacterial count exceeding 30,000 per c.c.	Percentage error of inclusion. Estimated on the total number of samples placed in the class	Number of samples of milk excluded from the class. Certified standard	Percentage error of exclusion. Estimated on the total number of samples of certified standard
hours					
5½	102	46	31·1	0	0
6	101	44	30·3	1	1
7	98	42	30	4	3·9
8	98	42	30	4	3·9
9	97	40	29·2	5	4·9
10	97	32	24·8	5	4·9
11	93	20	17·7	9	8·8
12	90	10	10	12	11·8
13	86	6	6·5	16	15·7
14	81	4	4·7	21	20·6
15	78	4	4·9	24	23·5
16	74	2	2·6	28	27·5
17	68	2	2·9	34	33·3
18	63	1	1·6	39	38
19	56	1	1·8	46	45
20	51	0	0	51	50

In Table VI the results for milk of Certified standard have been analysed. The second column gives the number of samples of milk of this grade that would be included in a class if the length of time given in the first column were adopted as the reduction time for the class. The third column gives the number of samples of milk of a lower grade (*i.e.* Grade "A" and Commercial) that would also be included. The percentage error of inclusion has been estimated on the total number of samples that would be included in the class if

the corresponding time limit were adopted; *i.e.* if 12 hours were taken as the minimum reduction time for Certified milk, then, of 100 samples placed in this grade, 10 would be incorrectly included, an error of 10 per cent. The fifth column gives the number of samples of milk of Certified standard that would be excluded. The percentage error of exclusion is estimated on the total number of samples of Certified standard; *i.e.* if 12 hours were again regarded as the minimum reduction time for Certified milk, then of 102 samples, 12 would be excluded, an error of nearly 12 per cent.

The results for milk of Grade "A" standard have been analysed in a similar manner in Table VII.

Table VI gives no evidence that a reduction time can be selected which could be adopted as a satisfactory limit for the grading of Certified milk. If, for example, 14 hours were adopted as the minimum reduction time, then the error caused by the inclusion of milk of a lower grade would be small, *i.e.* less than 5 per cent., but the error caused by the exclusion of samples of Certified milk would be great, *i.e.* more than 20 per cent. Conversely, if 10 hours were taken as the minimum, then the error of exclusion would be small, *i.e.* less than

Table VII.

Reduction time to be regarded as minimum for the class	Number of samples of milk *included in the class*: Grade "A" standard. Bacterial count exceeding 30,000, but not exceeding 200,000 per c.c.	Number of samples of milk *included in the class*: Commercial standard. Bacterial count exceeding 200,000 per c.c.	Percentage error of inclusion. Estimated on the total number of samples placed in the class	Number of samples of milk excluded from the class. Grade "A" standard	Percentage error of exclusion. Estimated on the total number of samples of Grade "A" standard
hours					
$5\frac{1}{2}$	25	21	45·7	1	3·8
6	25	19	43·1	1	3·8
7	25	17	40·5	1	3·8
8	25	17	40·5	1	3·8
9	24	16	40	2	7·7
10	21	11	34·4	5	19·2
11	14	6	30	12	46·1
12	8	2	20	18	69·1
13	5	1	16·6	21	80·8
14	3	1	25	23	88·5
15	3	1	25	23	88·5
16	1	1	50	25	96
17	1	1	50	25	96
18	1	1	50	25	96
19	1	0	0	25	96
20	1	0	0	25	96

5 per cent., but the error of inclusion would be correspondingly great, *i.e.* nearly 25 per cent. If 12 hours were taken as the minimum, then the errors of exclusion and of inclusion would be practically equal, but an error of 10 per cent. is too great for a method which is to serve as a just basis of payment or of legal procedure.

With Grade "A" milk the difficulty of selecting a satisfactory limit for the reduction time for the grade is even greater. But too much emphasis should

not be laid on Table VII, since only 26 samples are considered, and of these 3 only were being sold as Grade "A" milk. The remainder were samples of ordinary unlicensed milk of commerce.

In Table VIII the samples of milk have been grouped according to the numbers of samples of each grade that would be included or excluded if the reduction time given in the first column were taken as the minimum for a class. For instance, if 10 hours were adopted as the minimum for a class, this class would contain 97 samples of milk which were within the Certified standard, 21 samples within the Grade "A" standard and 11 samples beyond either of

Table VIII.

Reduction time to be regarded as minimum for the class	Number of samples of milk included in the class			Number of samples of milk excluded from the class	
	Certified standard. Bacterial count not exceeding 30,000 per c.c.	Grade "A" standard. Bacterial count exceeding 30,000 per c.c., but not exceeding 200,000 per c.c.	Commercial standard. Bacterial count exceeding 200,000 per c.c.	Certified standard	Grade "A" standard
hours					
5½	102	25	21	0	1
6	101	25	19	1	1
7	98	25	17	4	1
8	98	25	17	4	1
9	97	24	16	5	2
10	97	21	11	5	5
11	93	14	6	9	12
12	90	8	2	12	18
13	86	5	1	16	21
14	81	3	1	21	23
15	78	3	1	24	23
16	74	1	1	28	25
17	68	1	0	34	25
18	63	1	0	39	25
19	56	1	0	46	25
20	51	0	0	51	26

these two categories, while 5 samples of Certified standard and 5 samples of Grade "A" standard would be excluded. From this table it would appear that the Fermentation-Reductase Test does not offer a satisfactory means of discrimination between Certified, Grade "A" and ordinary Commercial milk, even by a subdivision of Class I on Orla-Jensen's scale. No two reduction times can be selected which will differentiate between the three grades.

If, however, the reduction time of a sample of milk were found to be long, *e.g.* 16 hours, that sample could with comparative safety be regarded as of Certified standard, for of the 76 samples recorded as having a reduction time of 16 hours or more, 2 only were below Certified standard bacteriologically. If, again, the sample had a short reduction time, *e.g.* less than 6 hours, the sample could, with only a slight chance of error, be regarded as below both Certified and Grade "A" standards, for of the 102 Certified and 26 Grade "A" samples recorded, 2 only had a reduction time of less than 6 hours. If the reduction time of a sample were found to be between the limits of 6 and 16

hours, it would be impossible to grade it with any degree of certainty by the use of this test alone. This is made very clear in Table VIII from which it is evident that for any reduction time between 6 and 11 hours there would be included in the class not only milk of Certified standard, but also many samples which were, bacteriologically, below this standard. On the other hand, as the minimum reduction time is prolonged, it is found that an unjust and increasing proportion of samples of milk of Certified standard would be excluded. The relationship between the bacterial counts and the reduction time was found to be very erratic for milk of low bacterial content. Two typical cases may be quoted:

(i) 32 samples of milk had bacterial counts of not more than 1000 per c.c., but the corresponding reduction times varied from 49 to $6\frac{1}{4}$ hours.

(ii) 2 samples of milk from the same farm, but tested on different days, gave the following results:

(*a*) Bacterial count 60,000 per c.c. Reduction time $19\frac{1}{2}$ hours.

(*b*) Bacterial count 60 per c.c. Reduction time $18\frac{1}{2}$ hours.

The question of the assistance that this test might offer to those who are more particularly interested in factory problems is rather outside the scope of this paper. Other workers have commented favourably on its use in this connection since it affords a rapid means of classifying milk into "good," "bad" and "indifferent." But the results for samples of ordinary Commercial milk which were discussed in Part II of this paper (cf. Table IV) suggest the advisability of first ascertaining whether Orla-Jensen's scale, which was drawn up as the result of a series of tests on milk from Scandinavian countries, can be applied without modification for the classification of the particular types of milk examined at any given factory.

The fact that the Fermentation-Reductase Test does not afford a satisfactory method for differentiating between samples of milk having a low bacterial content is not surprising when the nature of the test is considered. The reduction of the methylene blue is not a simple bacterial action. As Barthel (1917, p. 142) has shown, other factors are concerned, *e.g.* the reducing power of the milk itself, the amount of oxygen dissolved in the milk, the presence of leucocytes—and each has an influence on the reduction time of the milk. It has also been demonstrated by Orla-Jensen (1907, p. 222) and Fred (1912, p. 402) that the rate of reduction of methylene blue varies with different types of bacteria. It is contended that in the mixed milk of commerce these factors are negligible, and that if the samples are always saturated with oxygen by means of a thorough shaking before testing, the reduction time does afford an approximate measure of the number of bacteria present in the milk. This may be true for samples of milk in which the bacterial content is high enough to outweigh all other considerations, especially when certain types of bacteria commonly present in milk, *e.g.* lactic acid bacteria and *B. coli*, are dominant. But in milk of a better quality, in which the bacterial count is low, it is conceivable that these factors (*e.g.* the reducing power of

milk itself, the reducing power and optimum temperature of growth of the dominant bacteria, the influence of the various types of cells that are present, etc.) assume a relatively great importance, and must therefore be taken into consideration if this test is to be applied to such milk.

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