THE MILK SUPPLY OF THE CITY OF EDINBURGH.

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The objects of the investigations described in this paper were:

(1) To obtain data on the bacteriological quality of the milk supply of the city of Edinburgh;

(2) To compare such data with similar information for other cities;

(3) To demonstrate the value of certain biochemical tests for milk, at present little used in this country.

In the past a large amount of investigational work has been carried out along the general lines of the present enquiry. But frequently too much stress has been laid on the total amount of the bacterial contamination of milk and too little attention paid to its quality. A milk containing several hundreds of intestinal lactose-fermenting organisms per c.c. is much more likely to have injurious effects on the consumer than one containing several millions of normal lactic acid bacteria in the same quantity. Thus, it is of considerable importance to have a method for the examination of milk which will rapidly and fairly accurately demonstrate the nature of the bacterial contamination. The milk fermentation test, which will be described in this paper, fulfils these requirements.

In milk investigations and other work account must also be taken of the quantity of bacterial contamination. This is generally determined by plating known dilutions on ordinary or whey agar. Results are not obtained much inside a week and a considerable amount of special apparatus is required. The reductase test from which results can be got in a few hours is much more convenient and gives sufficiently reliable indications of the quantity of bacterial contamination.

METHODS.

Sampling. The samples used in this work were taken in small sterile milk cans. The can was taken to the milk shop and the milk bought under the same conditions as applied to sales to the general public. The samples were immediately taken to the Laboratory and examined.

The following tests were carried out for each sample:

(1) *Determination of Bacterial Content.* Counts were made in the usual way by the dilution method on whey agar at 22° C. (incubated for 10 days). Powdered chalk was added to the medium before it solidified to distinguish acid-producing colonies. The acid formed by the latter dissolves the chalk

in their neighbourhood: thus acid-forming colonies are surrounded by a clear zone in the otherwise turbid medium.

(2) *Determination of Content of Lactose Fermenters.* Known dilutions of the milk were inoculated into bouillon containing 1 % lactose in test tubes, each containing a fermentation tube. The bouillon was incubated at 37° C. for three days and the presence or absence of gas formation in the various dilutions noted.

(3) *Reductase Test.* This test depends on the power, possessed by all bacteria, of reducing and decolourising solutions of certain stains. Thus the higher the bacterial content of a milk sample the more quickly does it decolourise the stain. 40 c.c. of the milk sample were taken in a sterile boiling-tube and 1 c.c. dilute methylene blue solution (5 c.c. saturated alcoholic solution stain + 195 c.c. distilled water) added. The tube was incubated at 37° C. and the time required for the decolourisation of the stain noted.

(4) *Fermentation Test.* 40 c.c. of the milk sample were placed in a sterile boiling-tube and incubated for 24 hours at 37° C. The resulting curd is a good guide to the nature of the bacterial contamination. The following are the chief types:

(*a*) Milk remains liquid: contains few bacteria, generally only micrococci from the inside of the cow's udder, which have little action on milk.

(*b*) Curd gelatinous and uniform with no whey: sample contains chiefly true lactic acid organisms.

(*c*) Curd gritty: sample contains considerable numbers of true lactic acid bacteria and also sporing rods from dirty milk vessels, dust, air, etc.

(*d*) Curd cheesey: sample contains mainly sporing rods.

(*e*) Curd blown: abundant gas formation: milk contains large numbers of intestinal, lactose-fermenting organisms.

A good quality milk should give a curd of type (*a*) or (*b*): i.e. it should contain only organisms to be regarded as normal to milk. Contaminated milk will give a gritty, cheesey or blown curd. The last named is to be regarded as the worst: next comes the cheesey type, followed by the gritty which may be only slightly inferior to the gelatinous.

(5) *Catalase Test.* Milk possesses the power of liberating oxygen from hydrogen peroxide. The constituents of milk responsible for this change are the bacteria and enzymes and the animal cells derived from the inside of the cow's udder. But all the bacteria in milk cannot liberate oxygen from hydrogen peroxide: the most notable exceptions are the true lactic acid bacteria. 10 c.c. of the milk sample were mixed with 5 c.c. hydrogen peroxide (1 %) and the gas evolved during 24 hours at 37° C. measured. Koestler's apparatus was employed.

(6) *Sediment Test.* 10 c.c. of milk were placed in a centrifuge tube and warmed to 60° C. The tube was then centrifuged for 5 minutes. Observations were made on the quantity, colour and nature of the sediment from which a film was made and stained with aqueous methylene blue.

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DISCUSSION OF RESULTS.

The results obtained from the examination of 40 samples of Edinburgh milk are shown in Table I.

Table I.

No. of sample	Bacteria per c.c.	Acid producers per c.c.	Acid producers %	Non-acid producers per c.c.	Non-acid producers %	Reduced in hrs.	Lactose Fermenters per c.c.	Lactose Fermenters %	Fermentation test: Gas formation	Fermentation test: Type of curd	Catalase c.c. oxygen
1	270,000	130,000	48	140,000	52	>5½	500	·185	*g**	Sl. gritty	—
2	350,000	20,000	6	330,000	94	>5½	1	·000	—	Liquid	1·7
3	100,000	35,000	35	65,000	65	>5½	500	·500	—	Sl. gritty	2·4
4	29,000,000	26,000,000	90	3,000,000	10	4	10,000	·034	*B*†	—	2·5
5	20,000	<10,000	<50	>10,000	>50	>5½	500	2·500	*B*	—	1·9
6	280,000	230,000	82	50,000	18	>5½	100	·036	*B*	—	2·5
7	160,000	50,000	31	110,000	69	4	5,000	3·125	*B*	—	3·2
8	120,000	50,000	42	70,000	58	>5½	10	·008	—	Cheesey	1·9
9	310,000	280,000	90	30,000	10	>5½	10	·003	—	Gritty	2·1
10	130,000	30,000	23	100,000	77	>5½	10	·008	*g*	Cheesey	1·9
11	90,000	30,000	33	60,000	67	>5½	10	·011	*B*	—	2·4
12	3,300,000	160,000	5	3,140,000	95	4½	100	·003	*g*	Cheesey	2·1
13	280,000	70,000	25	210,000	75	>5½	100	·036	*g*	,,	3·2
14	230,000	150,000	65	80,000	35	>5½	10	·004	—	Gritty	1·9
15	800,000	500,000	62	300,000	38	>5½	100	·012	—	,,	2·6
16	165,000	80,000	48	85,000	52	>5½	10	·006	—	,,	1·9
17	210,000	70,000	33	140,000	67	5½	100	·048	*g*	,,	2·1
18	600,000	90,000	15	510,000	85	>5½	10	·002	*g*	Cheesey	1·2
19	320,000	70,000	22	250,000	78	4	1,000	·312	—	,,	2·0
20	160,000	40,000	25	120,000	75	>5½	100	·063	*B*	—	1·5
21	170,000	60,000	35	110,000	65	>5½	1	·000	—	Gritty	1·9
22	60,000	15,000	25	45,000	75	>5½	10	·017	—	Cheesey	1·4
23	600,000	350,000	58	250,000	42	>5½	—	·000	—	Sl. gritty	3·7
24	390,000	140,000	36	250,000	64	>5½	1	·000	—	Cheesey	2·25
25	3,050,000	1,000,000	33	2,050,000	67	3¼	100	·003	*g*	,,	4·8
26	1,500,000	180,000	12	1,320,000	88	4	1,000	·067	*B*	—	4·5
27	960,000	400,000	42	560,000	58	>5½	10	·001	—	Cheesey	2·1
28	280,000	140,000	50	140,000	50	>5½	100	·036	*B*	—	1·5
29	29,000,000	1,000,000	3	28,000,000	97	2¾	10	·000	—	Cheesey	2·6
30	420,000	190,000	45	230,000	55	>5½	1,000	·238	*B*	—	1·2
31	450,000	250,000	56	200,000	44	5	1,000	·222	*B*	—	2·1
32	9,300,000	9,200,000	99	100,000	1	4	1,000	·011	—	Sl. gritty	3·9
33	12,500,000	7,500,000	60	5,000,000	40	3¼	10	·000	—	Gritty	2·1
34	120,000	35,000	29	85,000	71	>5½	100	·083	—	Sl. gritty	1·7
35	1,250,000	700,000	56	550,000	44	>5½	10	·000	—	,,	4·6
36	450,000	200,000	56	250,000	44	>5½	50	·011	—	,,	5·0
37	155,000	130,000	84	25,000	16	5	—	·000	*B*	—	6·3
38	160,000	70,000	44	90,000	56	>5½	—	·000	*g*	Cheesey	4·6
39	125,000	15,000	12	110,000	88	>5½	—	·000	—	,,	6·4
40	60,000	500	1	59,500	99	>5½	1	·002	—	,,	3·9

* *g* = slight gas formation. † *B* = blown curd.

Bacterial Counts. The bacterial content of the samples examined varied from 20,000 to 29,000,000 per c.c. or just over 2,500,000 on the average. But an average of figures varying so widely does not convey much: the few samples with a content of over 1,000,000 were in many cases badly contaminated and so greatly raised the average. A better and fairer idea of the extent of the bacterial contamination is got by classifying the counts. Four of the samples (10 %) contained fewer than 100,000 organisms per c.c.; 24 (60 %) gave counts varying from 100,000 to 500,000; four (10 %) gave results between 500,000 and 1,000,000 and eight samples (20 %) showed more than 1,000,000 bacteria per c.c. Thus the majority of the samples show counts varying from 100,000 to 500,000 per c.c.

These figures, when compared with those for other cities, do not appear to be abnormally large—though they could be greatly lowered by the introduction of better methods of production and handling of milk. Table II (1) shows

Table II.

Year	City	Bacteria per c.c.
1885	Amsterdam	2,500,000– 10,500,000
1889	Munich	200,000– 6,000,000
1889	Würzburg	1,000,000– 2,000,000
1891	Halle	6,000,000– 30,000,000
1892	Giessen	83,100–169,632,000
1892	Würzburg	1,200,000– 7,200,000
1893	Dorpat	2,000,000–117,000,000
1894	Christiania	160,000– 45,000,000
1895	Petrograd	400,000–115,300,000
1895	Middletown	11,000– 8,500,000
1898	Guelph	121,000– 1,200,000
1898	Königsberg	12,500– 21,500,000
1899	Helsingfors	20,000– 34,300,000
1900	Dresden	250,000– 5,478,000
1901	New York	250,000– 30,000,000
1904	Middletown	8,000– 3,000,000
1905	Madison	35,000– 2,000,000
1905	Raleigh	34,000– 6,768,000
1905	Moscow	100,000– 7,000,000
1907	Raleigh	1,200– 54,000,000
1907	London	20,000– 8,000,000
1907	Berlin	43,000– 11,500,000
1907	Munich	204,000– 4,251,000
1907	Budapest	52,800– 18,000,000
1909	Chicago	10,000– 18,000,000

corresponding data for 25 other cities—from results published between 1885 and 1909. Since that date the main figures published are summarised below:

Table III.

Year	City	Bacteria per c.c.	Authority*
1910	Copenhagen	400,000– 32,000,000	(2)
1910	Lisbon	73,000–271,000,000	(3)
1911	St Paul, Minn.	Average = 8,206,000	(4)
1911	Chicago	10,000–100,000,000	(5)
1912	Leipzig	27,500–142,000,000	(6)
1912	Washington	Under 500,000 = 35%: over 500,000 = 65%	(7)
1912	New York	,, 100,000 = 83%: over 100,000 = 17%	(8)
1913	Moscow	13,000– 82,500,000	(9)
1917	Geneva, N.Y.	Under 200,000 = 88%: over 200,000 = 12%	(10)

* See numbered references at end of this Paper.

It will be seen that the figures obtained here compare quite well with those got for other cities—with the exception of New York where the milk supply is particularly clean. But it is well to bear in mind that many of the figures quoted apply to samples taken throughout the whole year, whereas, in the present enquiry, we have dealt exclusively with milks obtained during the winter months. The low winter temperature would naturally favour low bacterial counts.

But though the figures obtained compare well with those for other cities it must not be supposed that the Edinburgh milk supply is particularly clean. As will be shown later the nature of the bacterial contamination is particularly bad. And, in addition, great reductions in total bacterial numbers will have to be made before the supply can be considered a really clean one.

Reductase Test. The standards suggested by Barthel and Jensen (11) for use with this test are as follows:

Quality of milk	Time required for reduction	Bacteria per c.c.
Good	Longer than 5½ hrs.	About 500,000
Medium	2–5½ hrs.	500,000– 4,000,000
Poor	20 mins.–2 hrs.	4,000,000–20,000,000
Very poor	Under 20 mins.	Over 20,000,000

According to these standards 28 of our samples (70 %) would be classed as good and the remaining 12 (30 %) as medium. These figures correspond well with those given under "Bacterial Counts" where it is shown that 70 % of the samples gave counts under 500,000.

Relation between Bacterial Counts and Reductase Test. The time required for reduction corresponds fairly well with the bacterial counts except in a very few cases. Those samples which contained large numbers of bacteria have, in practically every case, shown a short reduction period: while generally where the numbers of bacteria present have been small the time required for reduction has been fairly long. But the bacterial counts and times of reduction do not correspond well with those laid down by Barthel and Jensen (11) (see above) and, as has been noted by Schroeter (6), the latter would appear to require some modification. Our results lead us to the conclusion that a milk sample which reduces within 5½ hours generally contains 1,000,000 or more bacteria per c.c. The only exceptions in the present investigation were samples 7, 19, 31, 35 and 37. The only case in which the reductase test failed to show a milk which contained more than 1,000,000 organisms per c.c. was sample 35, and here the bacterial content was just over 1,000,000 and the sample was otherwise a comparatively clean one (contained fewer than ·001 % of lactose fermenters). In the four cases in which the reductase test showed a short reduction period for samples containing fewer than 1,000,000 organisms per c.c., all are to be regarded as badly contaminated samples. Nos. 7, 19 and 31 contained a high percentage of lactose fermenters: No. 37 produced a blown curd in the fermentation test. It may be said, therefore, that in no case has the reductase test condemned a really clean sample and with one exception—and that an unimportant one—it has never failed to detect a sample containing more than 1,000,000 bacteria per c.c. when the standard is modified as above suggested.

Content of Lactose Fermenters. The content of lactose fermenters varied widely (from 0 to 10,000 per c.c.). 20 % of the samples contained lactose fermenting bacteria in numbers of 1 or under per c.c. of the milk; 30 % showed these organisms in numbers from 1 to 10 per c.c.; 25 % in numbers from 10 to 100 per c.c.; 20 % in numbers from 100 to 1000 per c.c.; 5 % in numbers from 1000 to 10,000 per c.c.

Various standards have been suggested for the numbers of lactose fermenters in clean milk. Vanderleck (12) holds that they should not constitute more than 1 % of the total bacterial count—a standard which appears to err on the side of leniency (cf. Schroeter). Savage (13) considers that in winter milk might be classified as follows with regard to its content of lactose fermenters:

Pass = under 100 per c.c.; Unsatisfactory = 100–500 per c.c.; Condemn = over 500 per c.c. Kinyoun (14) found that the average for good milk was 1 lactose fermenter per 50,000 other bacteria (·002 %) while dirty milk contained 1 lactose fermenter per 555 others (·180 %). Ayers, Cook and Clemmer (15) found lactose fermenting organisms in ·01 c.c. of milk in a relatively small number of samples.

From the data given by Savage and by Ayers, Cook and Clemmer it seems not unreasonable to expect that in clean milk not more than 10 lactose fermenters per c.c. should be found. In the samples examined by us which showed lactose fermenters in numbers up to 10 per c.c. none gave a percentage of these organisms in the total count higher than ·02—a figure which corresponds well with the data given by Kinyoun.

If one takes the standard of 10 lactose fermenters per c.c. it is found that 20 samples (50 %) would be condemned. Taking the standard as ·02 % of the total count, 15 samples (37·5 %) fail to satisfy the requirement. Both of these percentages are abnormally high and indicate either very considerable original contamination with undesirable organisms or great multiplication of these organisms in the milk on keeping. But the organisms of the intestinal group require a high temperature for their activity—somewhere in the neighbourhood of 37° C. And as the air temperature during the period covered by these experiments was very low and the samples were generally found to be well cooled in the retailers' premises, one is forced to the conclusion that the responsibility for the high counts rests mainly on the producer. The high figures for lactose fermenters would appear to be due to insufficient attention to cleanliness in the production of the milk.

Fermentation Test. The results of this test are given in two columns headed "Gas formation" and "Type of curd." In the former only those samples which produced gas are noted: in the latter those which did not produce blown curds. Eleven samples (27·5 %) gave blown curds while eight

(20 %) produced small quantities of gas in the test. These results are far from satisfactory and support those of the counts of lactose fermenters in emphasising the undesirable nature of the bacterial contamination. They show that about half of the samples contain lactose fermenters in dangerously high numbers.

There is no very marked agreement between the formation of gas in the fermentation test and the number of lactose fermenters per c.c. or the percentage of the latter in the total bacterial count. Generally, however, the badly contaminated milks have produced blown curds. Further the test is probably a fairer one both from the public health and dairying points of view than simple counts of the lactose fermenters because:

(i) a large quantity of milk is examined (40 c.c.),

(ii) it is carried out in the milk itself.

In those cases where the gas-forming organisms have taken the upper hand in the fermentation test they probably would have done so if the milk had been consumed or if it had been made into cheese.

Of the 29 samples which did not show a blown curd in the fermentation test only one remained liquid: 14 gave gritty curds and 14 cheesey curds. The sample which remained liquid (No. 2) contained a very low percentage of acid-producing organisms and the non-acid producers present evidently had little action on milk. None of the samples examined gave an absolutely uniform gelatinous curd. Thus according to the results of the fermentation test only one sample could be considered to be really first class.

Milks which produce a gritty curd are to be regarded as fairly satisfactory. The proportion (35 %) in this case is decidedly low, however, when one remembers that only one sample can be classed in types 1 and 2. In the gritty samples the percentage of non-acid producers varies from 1 to 71.

Samples which give a cheesey curd are generally highly contaminated with spore-bearing organisms from fodder, soil, air, improperly sterilised milk vessels, etc. 35 % of the samples showed cheesey curds. These samples contained 56–99 % of non-acid producing organisms.

Thus of the 40 samples examined, 14 produced cheesey and 11 blown curds. Both types are unsatisfactory and indicate high contamination. Together they make up 62·5 % of the samples investigated—a proportion which is unduly high.

Catalase Test. The figures for the catalase test vary widely and no relation could be found between them and the other results.

Sediment Test. The amount of sediment present in the samples only exceeded ·1 % in one case (No. 3). Generally the sediment was brownish in colour and streptococci, rods and cells could frequently be seen in it.

SUMMARY AND CONCLUSIONS.

In tests of 40 samples of milk from the supply of the city of Edinburgh:

(1) The bacterial content was, on the average, high but not abnormally so when compared with that of the milk supply of other cities. It ranged from 20,000 to 29,000,000 per c.c.

(2) The results of the reductase test corresponded well with the bacterial counts and the test is recommended for obtaining a good rough idea of the bacterial numbers present, where results are required in a short time.

(3) About half of the samples contained lactose-fermenting organisms in unduly high numbers; this is attributed chiefly to the lack of attention to cleanliness in production.

(4) The value of the fermentation test for speedily indicating the nature of bacterial contamination is pointed out. In a general way the test corroborated the results of counts of the lactose fermenters and indicated that a number of the samples, which did not produce blown curds, contained large numbers of undesirable organisms.

(5) Taken as a whole, the results seem to indicate not so much particularly high contamination as contamination of a very undesirable nature. They point to the necessity of educating the producer and distributor in the principles underlying the production of clean milk and emphasise the importance of proper control of the industry from this point of view.

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