MILK AND ELECTRICAL DISCHARGES.

*By Professor W. G. DUFFIELD, D.Sc., and
Mr. J. A. MURRAY, B.Sc.*

The experiments to be described originated from a curiosity to test the popular belief that the electrical conditions during a thunderstorm are responsible for the rapid souring of milk.

Our method was to pass air containing the products of an electric spark discharge through milk, and to observe the effect upon the rate at which the acidity increased; the

results demonstrate that in so far as our experimental arrangement imitated the electrical conditions of a thunderstorm—probably not very closely—it should tend to preserve milk rather than to turn it sour, the associated temperature conditions being presumably responsible for the increased acidity observed on these occasions.

The experiments were subsequently continued on account of their bearing upon dairying. The first series of experiments were carried out in the Physical Laboratory of University College, Reading, in June and July, 1912.

A second series was undertaken about a week ago (on November 26th, 1913) and the results proved confirmatory of the first set.

As far as we have been able to discover milk has not been previously treated in this way. In 1911 (British Association Report, Portsmouth, 1911), Priestley and Lee passed small currents through an electrolytic cell containing a milk culture of *Bacillus Bulgaricus* and measured the change in its resistance. The acidity, as measured by its resistance increased as the current increased until a maximum was reached at about 60 micro-amperes, when a bacteriological count indicated a maximum number of growths; a further increase to 80 micro-amperes at 40°C. in the current was found to produce an inhibitory effect. In the same year Thornton*

exposed Agar plates inoculated with various Bacilli (*B. Anthracis*, *Pneumococcus*, *Coli communis*, *typhosus*, etc.) to a brush discharge from a unidirectional high tension source and observed a marked sterilizing action which he attributed to the ions formed in the air, but which Priestley and Knight† from observations upon *B. Coli Communis*, ascribe to the chemical products of the discharge. An interesting feature of Thornton's work was the predominance of the effect of the negative over that of the positive pole.

The present experiments were conducted with the apparatus shown in Fig. 1, the tube containing glass-wool or cotton-wool being omitted from all save the filtration experiments, which are described at the end of the paper. They were performed in the following manner:—

A discharge between the two brass points 4 cms. apart was produced by an induction coil, the electrodes being contained in a wide glass tube 4 cms. in diameter fitted with perforated corks, and through this tube air was sucked by means of an aspirator or a water pump. The products of the discharge could thus be carried with the air through a sample of milk which was contained in a large test-tube or small flask fitted with the usual cork and delivery tubes. A similar vessel was placed on the other side of the discharge tube and contained milk to serve as a control. Before the air from the room

* Proc. Roy. Soc. Series B, Vol. 84, p. 280, 1911.

† Proc. Roy. Soc. Series B, Vol. 86, 1913.

The Tube containing Glass Wool was only included in the experiments upon Filtration of the Products of the Discharge.

Fig. 1.

reached this it was passed through a plug of cotton-wool to prevent contamination from the air; a tube containing caustic potash was sometimes introduced, but it was not found to affect the results in any way, and was subsequently discarded. To prevent churning the bubbling was very slow, except in the last experiments.

After the discharge had passed for about an hour the treated milk and the control were divided into portions of 10 cc. each, which were tested at intervals by titration with decinormal caustic soda, using phenolphthalein as indicator.

A diagram representing the normal increase of acidity of milk with time is shown below which was plotted from the results of an

Diagram I. from Table I.

experiment quoted in Table I. obtained from the examination of milk which had acted as a control.

TABLE I.
Date—July 18th, 1912.

Interval in hours.	Acidity.	Temp. of Room.
0	2.1	
10	2.1	16° C.
20	2.1	
30	3.5	18° C.
42	7.6	18° C.
48	8.2	20° C.
54	8.5	19° C.
69	9.3	22° C.
75	9.8	22° C.
93	10.3	22° C.

The increase with time is at first slow, but a stage is reached at which the acidity increases rapidly; this is followed by a period during which the acidity is practically constant. The explanation of this

phenomenon which is usually given is that the increased acidity inhibits the action of the bacteria. A factor which appears to affect both the rate of increase of acidity with time (and possibly also the value of the maximum acidity which can be obtained) is the temperature, the higher the temperature the greater the rate of increase of acidity. The upward slope of the curve in the diagram after 60 hours, may be due to rise of temperature during the latter part of the observations. In subsequent experiments the temperatures are not quoted because the control and treated samples were always kept side by side and were at the same temperature.

The following are the results of three typical experiments:—

The figures are the number of cc. of alkali necessary to neutralise 10 cc. of the milk, and are hence measures of the acidity of the samples at the specified interval of time after the conclusion of the discharge.

If we assume that the whole of the acidity is due to lactic acid (of which we have no proof), the amount of lactic acid present may be calculated from the fact that 1 cc. of alkali corresponds to 0.009 grams of lactic acid.

TABLE II.

Date—June 12th, 1912.

Duration of Discharge, 52 mins.

Interval in hours between treatment and test.	Acidity of control.	Acidity of treated sample.
0	2.0	2.0
16	2.1	2.0
40	2.8	2.1
64	9.0	5.9

TABLE III.

Date—June 26th, 1912.

Duration of Discharge, 80 mins.

Interval in hours between treatment and test.	Acidity of control.	Acidity of treated sample.
0	2.0	2.0
22	3.3	2.0
46	8.3	3.2
120	9.6	9.6

TABLE IV.

*Date—July 18th, 1912.**Duration of discharge, 80 mins.*

Interval in hours between treatment and test.	Acidity of control.	Acidity of treated sample.
0	2.2	2.2
10	2.0	2.0
20	2.0	2.0
32	2.0	2.0
38	2.1	2.1
44	2.2	2.2
59	7.1	2.4
65	8.5	2.6
83	—	5.6

The results of the last two treatments are shown in Diagram II., and are marked A and B respectively.

Diagram II. from Tables III. and IV.

No immediate change in the acidity was observed, but after about 48 hours there was always a more rapid increase in the acidity of the control than in that of the treated sample.

These experiments suggest that there is some sterilizing action upon the milk through the agency of the electric discharge. From Table III. it will be seen that after a long interval the acidities of the control and treated sample are the same; this may be due to contamination when the milk was being divided into successive portions or to the sterilization due to the discharge being incomplete.

A different result was obtained when a stronger spark was used and more vigorous bubbling permitted.

TABLE V.

*Date—Nov. 26, 1913.**Duration of discharge, 71 mins.*

Interval in hours between treatment and test.	Acidity of Untreated Milk.	Acidity of Control Milk.	Acidity of Treated Milk.
0	1.9	—	—
20	—	—	—
38	5.0	4.3	2.6
46	7.5	7.4	3.0
52	8.3	8.0	3.2
63	9.3	9.5	3.8
70	9.6	9.8	3.8
76	9.6	10.0	4.0
87	10.1	10.3	3.9
97	10.3	10.4	4.0

Diagram III. shows that the acidity of the treated sample is now practically constant after about 63 hours and much less than that

Diagram III. from Table V.

of milk used as a control, the increase in the acidity of the former being but one quarter that of the latter. It suggests that the air which had passed through the discharge tubes had destroyed those bacteria which can exist in a high percentage of acidity, and that a selective action has been exercised. If a single bacterium capable of lactic acid formation had survived in the milk when an acidity of 3.8 was recorded, it is almost inevitable that a marked increase of acidity would have been observed in the treated sample between the 63rd and 97th hours. It occurred to us that the spore-bearing bacteria might have been annihilated, but a preliminary bacteriological examination indicated that some of this type still existed

after treatment. There is the further question whether the action is directly germinal or whether the medium in which the bacteria subsist has been so altered that certain of them cease to be effective acid producers.

In the second column of the experiment under discussion will be found the acidities of a sample of the same milk which was not treated in any way. It served as a check upon the control, and the results show conclusively that the difference in the acidities of the control and treated sample was due to a retarded formation of acidity in the latter, and not to an acceleration in the former.

It was found that the milk which had been treated remained uncurdled, even after 121 hours, though that in the control was very thick and curdled.

Experiments were performed to ascertain whether the nature of the action was in any way altered by decreasing the time during which the discharge lasted. From analogy with the action of drugs upon organisms some stimulating action might have been inferred before the dose became toxic. Exhaustive tests were not carried out, but no indication of stimulation of the acid-forming propensities was found, though a dose sufficiently small to give practically no effect in either direction was employed. The data are given in Table VI. which is illustrated by Diagram IV.

Diagram IV. from Table VI.

TABLE VI.

Date—Nov. 26th, 1913.

Interval in hours between Treatment and Test.	Acidities.			
	Control.	1 min.	5 mins.	71 mins.
0	—	—	—	—
20	—	1.9	1.9	—
38	4.3	4.3	2.8	2.6
46	7.4	7.9	4.2	3.0
52	8.0	8.4	4.9	3.3
63	9.5	9.1	6.8	3.8
70	9.8	9.6	8.3	3.8
76	10.0	9.9	9.4	4.0
87	10.3	10.2	9.3	3.9
97	10.4	—	—	4.0

The curve for the 5 minutes exposure has been drawn with a flat maximum since the figures indicate that the acidity is constant after 76 hours; this is further support of the idea that the action is selective, and that those bacteria which are normally capable of existence in highly acid milk are the first to succumb. The behaviour of the milk quoted in Table III. appears to negative this, but it is possible that in that instance the whole of the bacteria of that type were not exterminated though a large proportion of them may have been affected.

We attempted to find whether the action is due to the electrical or chemical products of the discharge. Since the latter are chiefly ozone and oxides of nitrogen, with possibly some hydrogen peroxide, an experiment was carried out in which the air was first passed over a tube containing red-hot copper filings to remove the oxygen, but the nitrogen which remained was not pure, and we place no reliance upon the result of the experiment.

It seemed more satisfactory to cause the ions formed by the discharge to recombine by passing the air through a tube containing cotton or glass wool (shewn in fig. 1), before proceeding to the sample to be treated, when any sterilizing action would be produced presumably by the chemical products alone.

The first two experiments performed in this way are illustrated by the following tables and by Diagram V.

TABLE VII.

Date—July 3, 1912.

Duration of Discharge 62 mins.

Interval in hours between Treatment and Test.	Acidity of Control.	Acidity of Treated Sample (Filtered).
0	2.1	2.1
24	7.9	7.3
43	9.4	8.3
67	9.9	9.6

Tube of glass-wool used as filter

TABLE VIII.

Date—July 18, 1912.

Duration of discharge $2\frac{1}{2}$ hrs.

Interval in hours between Treatment and Test	Acidity of Control.	Acidity of Treated Sample (Filtered).
2	2.1	2.1
25	3.3	2.3
37	7.1	2.2
43	8.1	2.5
49	8.6	2.8

Tube of glass-wool used as filter.

Diagram V. from Tables VII. and VIII.

The first appears to show that the filtered gas has very little influence as an inhibitory agent, the second provides strong evidence that it is a powerful bactericide. A third experiment more recently performed shows that the difference lies partly in the state of the milk at the time of the experiment and partly in the duration of the treatment.

Data of the final experiment are given in Table IX. and the graph drawn from it in Diagram VI. The columns headed Control and Treated are taken from the experiment

Diagram VI. from Table IX.

already quoted (Table V.) which was carried out with the same milk on the same afternoon; the numbers in the final column were obtained from an experiment performed immediately after the other was concluded and differed only in the duration of the discharge which was slightly greater—82 mins. instead of 71 mins.

TABLE IX.

Date—Nov. 26, 1913.

Interval in hours between Treatment and Test.	Acidities.		
	Control.	Treated.	Filtered (tube of cotton wool used as filter).
38	4.3	2.6	2.2
46	7.4	3.0	3.0
52	8.0	3.2	3.2
63	9.5	3.8	4.4
70	9.8	3.8	5.3
76	10.0	4.0	7.1
87	10.3	3.9	7.5
97	10.4	4.0	7.9

Exposures—Treated 71 minutes.

Filtered 82 minutes.

During the first fifty hours the filtered sample increased in acidity at the same rate as the treated sample and less rapidly than the control, but during the second half of the experiment its acidity increased rapidly and was still increasing when the final sample was examined. It is interesting to compare it with the curves in Diagram IV., and it appears that it may be regarded as intermediate between the curves for 5 minutes

and 71 minutes, and of the same general shape, save that the acidity is increasing rather more rapidly 97 hours after the end of the treatment.

Since the rate of bubbling was the same with and without the tube of cotton-wool it seems that the filtration is capable of removing some of the sterilizing agent or agents. The similarity in shape already mentioned precludes the effects of the two types of agent from being greatly different from one another. Both prolonging the discharge and removing the filter are capable of destroying the bacteria which are already exposed to the rigours of a highly acid medium.

That ozone (or oxides of nitrogen) passed freely through the filter was shown by a starch iodide paper and it can scarcely be disputed that some of the inhibitory action is due to this product. The filtration should remove the ions formed by the discharge almost entirely and it is apparently their absence from the filtered air which has reduced the sterilization. The question arises (whether the action is due to ions or to ozone) whether the products of the discharge fulfil their sterilization by direct germicidal influence or indirect toxic action. In view of the impossibility of even a small proportion of the bacteria coming into contact with the air from the spark discharge without an intervening film of moisture, the latter is the view favoured and it is in agreement with the experiments of Priestley and Knight to which reference has already been made.

As it seemed to us very important to attack the problem from the bacteriological as well as the chemical standpoint, we were fortunate in obtaining the co-operation of Dr. Stenhouse Williams, Bacteriologist in the Dairy Department at University College, Reading.

We are indebted to him for testing samples taken from the Untreated, Treated, and Control milk used in the experiment,

whose data are given in Table V. in the following ways:—

- (a) To test for gas formation, glucose peptone water was inoculated and cultivated at 37° C. The treated sample showed no diminution in the amount of gas evolved, all samples showing acid or gas formations at the highest dilutions used, one thousandth of a c.c.
- (b) To test for organisms able to grow at 37° C, Agar plates were inoculated and cultivated at that temperature.
- (c) To test for the general bacteriological contents gelatine plates were inoculated and maintained at 22°C.

The results of (b) and (c) are given in the following Table:—

Agar.	Gelatine.	
Untreated, 161,000.	716,000	} Organisms in 1 c.c.
Control, 276,000.	446,000	
Treated, 160,000.	172,000	

The outstanding feature is the reduction in organisms in the Treated over those in the Control samples. There is no definite evidence that the air which has passed through the Control after filtration through the *first* plug of cotton-wool had any considerable effect upon the bacteriological contents, for although the growth upon Agar is greater in the Control than the Untreated, that upon Gelatine is less.

The counts were made from $\frac{1}{1000}$ th dilutions. In Plate 1, Figs. 1 and 2 and Figs 3 and 4, are shown the effects of the treatment upon the milk, the reduction in number of organisms in the treated samples, Figs. 2 and 4, being easily apparent at different dilutions. Some spore bearers are seen in both the Treated and Control dishes in Figs. 3 and 4. The samples above referred to were taken immediately after the conclusion of the treatment, but a further series was made by Dr. Williams with the samples which had been kept for 121 hours. The treated samples again show a great reduc-

tion in the number of organisms, but as those remaining include non-spore bearers it is unlikely that any large proportion of spore bearers have been destroyed.

The bacteriological evidence thus confirms the chemical tests that an inhibitory action had resulted from the passage of the products of the discharge through milk. From the last experiment the action appears to be by no means a transitory one since the bacteriological contents has remained markedly lower than that of the Control over a period of five days. The experiments are being continued more particularly from the bacteriological point of view.

We express our thanks to Dr. Stenhouse Williams for undertaking the bacteriological examination described below.

DISCUSSION.

DR. STENHOUSE WILLIAMS, the Bacteriologist who had examined the samples of treated milk, having been called on by the President, said the bacteriological part of the work had only started the previous week, and there must be many more experiments before a definite opinion can be arrived at. Obviously Professor Duffield has not destroyed all his non-spore-bearing organisms, and, that being so he is hardly likely to have destroyed his spore-bearing ones, which are very much more resistant. Whatever has happened has had a remarkable toxic or restraining effect upon the bacteria in the milk. The milk on the agar and gelatine plates (which were handed round for the inspection of Members) was milk which had been kept from Wednesday, when it was treated, until Monday morning before being bacteriologically examined. The treated samples had received 71 minutes' exposure to the electrical discharge, and the extent of dilution was the same for both the treated and the untreated milk. In the untreated milk innumerable colonies of micro-organisms

have developed, even in quantities as low as 0.000001 c.c.m., whereas in the milk treated by Professor Duffield on the Wednesday there are extremely few organisms present in any corresponding strength. The Control shown is diluted, 1 : 1,000, it is teeming with organisms, and Professor Duffield's corresponding sample is almost free. There is no question but that Professor Duffield has destroyed a very large number of organisms in this milk, and in addition that the destructive power, whatever its exact nature may be, has remained in the milk for at least five days after treatment.

MR. C. E. S. PHILLIPS asked if in the later experiments recorded in the paper a filter of cotton-wool was used. Professor Duffield's work seemed to negative the popular idea that thunderstorms turned milk sour.

DR. HORACE MANDERS suggested that much valuable information might be gained if experiments were made using various metals as electrodes; possibly some metals would be found more active than others.

DR. W. DEANE BUTCHER referring to the value of this method of sterilization from a medical point of view, asked for more information as to the kind and strength of the discharge used.

MR. W. DUDELL thought that electrical sterilization would be easy and not expensive but asked for more information as to the kind of discharge used; had Professor Duffield tried a brush discharge. The production of ozone was likely to play an important part in the matter, a study of the curves seemed in favour of this assumption.

DR. SIDNEY RUSS referred to similar work that had been carried out by Beattie and Lewis of Liverpool, and asked if Professor Duffield could say what were the organisms commonly found in milk.

MR. F. HARRISON GLEW suggested that the results might be explained on the assumption that the discharge between the metallic electrodes caused a portion of the metal to be thrown off in a colloidal state, and referred to the experiments of Mr. H. Crookes upon the bactericidal action of silver and other colloids; if this were so, filtering through cotton-wool should materially diminish the effect.

DR. HOWARD HUMPHRIS suggested that if ozone had been formed by the discharge, compounds of nitric acid would be formed also.

THE PRESIDENT: If there are no more members who wish to join in the discussion, perhaps before calling upon Professor Duffield to reply I may be allowed to make one or two remarks myself. Professor Duffield seems to think that with the experiments described in his paper he had got to the end of the chemical part of his inquiry. I venture to think, on the contrary, that this part of the inquiry is only just beginning. I was especially interested in the shape of the curves thrown on the screen. A great deal can be learned from the shape of these curves. Their appearance, to which attention was drawn, is what chemists call the bimolecular reaction, and the moment this bimolecular reaction is suggested we begin to know something definite as to what is proceeding. Take, for example, the case in which 71 minutes' exposure of the milk to the electrical discharge was given. I should like to ask whether the curve in that case begins after the end of the 71 minutes' treatment. I notice that the one characteristic of all the curves is that they start out horizontally. At the beginning the increase of acidity is nothing. That fact shows that whatever two things are coming together and undergoing this bimolecular reaction, one of them was absent at first, because the rate of the progress of the reaction at first

was zero. After a while the growth begins, so that whatever it was that was combining with the substance which was turning to lactic acid, it was something which was not present immediately after the 71 minutes' discharge, but which gradually grew afterwards, perhaps in consequence of the change brought about by the discharge, although not in evidence at first. I am fairly sure that there would be a great many other suggestive points if we were only to follow the curves out in detail. Those who are familiar with fitting on curves to chemical reactions—I have had some little practice in that way myself—might perhaps be able to give a hint or two in such researches as those carried out by Professor Duffield.

REPLY.

PROFESSOR DUFFIELD, in reply, said: I have been very much interested indeed in this discussion, and I am very glad indeed to have had two points brought forward. The first point, mentioned by two speakers, is with regard to the use of different metals for electrodes. This procedure would help us to arrive at some conclusions with regard to the possibility that we have a colloidal action between the metal that is torn off from the poles and the milk. I was ignorant of the experiments of Crookes upon that subject, but it is undoubtedly the case that we have particles torn off from the poles. The possibility of an action of a colloidal nature in this instance, however, had certainly not occurred to me. Some may remember a paper by Hemsalech describing how air which was drawn through a globe in which a spark discharge was passing between metal poles, was mixed with gas and passed into a burner, when the flame spectrum of the metallic electrodes appeared. It was further found that by introducing a plug of cotton-wool these charged metallic particles could be filtered out, when the spectral lines were no longer visible in the flame. The paper

by Crookes to which allusion has been made must have a very great bearing upon the experiments I have described, and I should be very glad to have the reference. I have been asked with regard to the size of the apparatus used in these experiments. I used a 15-inch coil, and the spark was 4 cms. long, but with the particular strength of current passed through the primary I could have got a spark about 9 inches long, so that it was a fairly "fat" spark. The particular poles we used in these experiments were of brass. The details of the size of the apparatus will be published in the printed paper. With regard to the brush discharge, Professor Thornton made use of it in his experiments. He blew the electric wind from the poles on to agar plates which had been infected with different bacteria, and he, as I have said, came to the conclusion that the effect was an electrical one, and that the negative pole was more effective than the positive. The positive was effective, but not to the same extent as the negative, and he thinks it possible that the whole effect is due to negative electrification, and that the certain small amount of negative electrification which comes from the positive pole is accountable for such positive effect as exists. I am glad to have the point brought out that the temperature effect upon ozone must be carefully watched. It is quite possible that this has something to do with the difference between the curves we got on different occasions. I have always had some difficulty, with Dr. Russ, in understanding how charged ions can act upon the bacteria directly. It is rather against a direct germicidal action when one remembers that these bacteria would be covered with a film of moisture, and that it would be difficult to expose them to the direct influence of the ionised air. But one might have ions producing some chemical change in the milk—not a germicidal action, but a toxic one. I have been asked as to the nature and number of organisms in milk. There are something like a hundred different kinds of

organisms in milk, and I may be excused from particularising them. The thunderstorm was not exactly reproduced, I admit, and I do not know how to do it. I do not press the point about the thunderstorm at all vigorously. The experiments of Priestly and Lee, in which on passing an electrolytic discharge through milk they got an increase of acidity, might be taken as acting contrary to our own investigations though not necessarily so. The current used by these investigators was very weak. We attempted to investigate the effect of small amounts of discharge by varying the time of exposure to it, thinking that it might have at first a stimulating and afterwards a killing effect upon the bacteria, but we have not observed any sign of stimulated activity as far as the production of acid is concerned. Professor Porter has raised another point of very great interest, that, namely, of the shape of the curve. But the character of the curve is the same as that which one gets with ordinary untreated milk. All the curves have the same general shape.* Any reasoning we adopt in interpreting them must be applied, not only to those representing the treated samples, but to those representing the untreated samples as well. One might conceivably regard the initial delay in the increase of acidity as being due to the time taken for the two factors which together produce acid to reach one another, for example, a barrier (such as a cell-wall) might have to be dissolved, broken down or penetrated before the contents of the cell could be reached and acid formation begin. I am extremely grateful for the hints which have been given me, which will be of very great value in continuing the work.

A vote of thanks was accorded by acclamation on the motion of the President.