THE ELECTRIC CURRENT (APART FROM THE HEAT GENERATED).

A BACTERIOLOGICAL AGENT IN THE STERILIZATION OF MILK AND OTHER FLUIDS.

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(With 2 Text-figures.)

Though we have, in former papers (1915, 1916, 1920), given details of the apparatus we used in producing sterilisation of milk by the electric current, it will be a convenience to readers to repeat some facts in regard to the arrangements of the electrodes and electrical circuit. The quantity of current used depends on the size of the apparatus, and the voltage is normally between 3000 and 4000. The alternating current is carried by the milk and is applied by means of three copper electrodes, $\frac{1}{8}$th of an inch thick, each enclosed in a glass electrode chamber.

The electrode chambers communicate with each other by means of stout glass tubing of even bore, the electrode holders and intermediate portions being continuous through joints

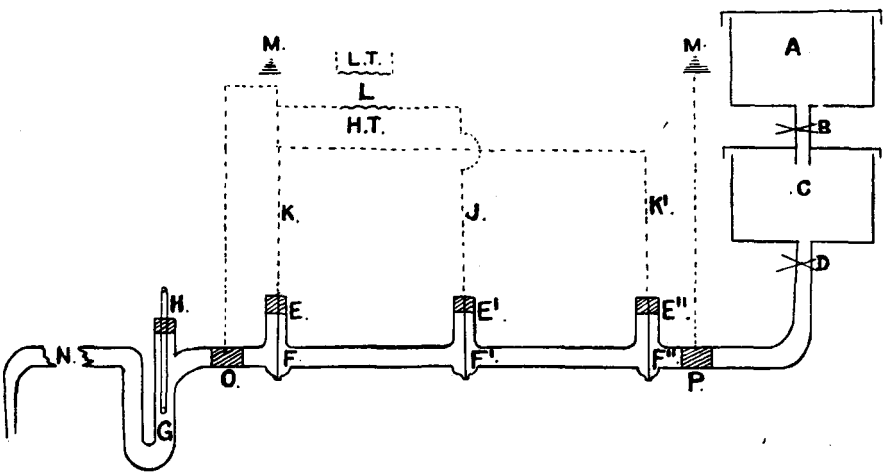

Fig. 1. *A*, covered receiving tank for untreated milk; *B* and *D*, taps; *C*, constant level tank; *E*, *E'* and *E''*, electrode chambers; *F*, *F'* and *F''*, electrodes; *O*, *P*, aluminium earthing tubes (the portion of the apparatus between *O* and *P* is the "Lethal tube"); *K*, *K'* and *J*, electric cables; *L*, transformer; *LT* and *HT*, low and high tension; *M*, earth connections; *H*, thermometer; *G*, thermometer holder; *N*, outlet pipe from lethal tube.

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of indiarubber tubing. This arrangement renders the whole apparatus somewhat flexible, and facilitates cleaning.

The sectional area of the "lethal tube" is strictly relative to the calculated output of the particular installation and the size of the electrode chambers and their enclosed electrodes is such as to present a minimal interference with the flow of milk. The superficial area of the electrodes is greater than the sectional area of the glass tube, hence no milk can pass through the apparatus without being submitted to the full action of the current.

In the commercially applied apparatus (Fig. 1) the diameter of the lethal tube was $\frac{3}{4}$ inch—the distance between the electrodes F and F' was 35 inches, and between F' and F'' was 20 inches. The rate of flow has to be carefully regulated (with this apparatus about 30 gallons of milk passed through in one hour), but in this regulation slight variations may require to be made in voltage and amperage in order to obtain the most efficient current density and terminal temperature. We depended very largely on the terminal temperature for establishing what may be regarded as standard conditions, for the reason that slight variations in the electrical conditions are more readily indicated by the sensitive thermometer than by the voltmeters and ammeters. Any variation in the temperature reading can be controlled by very slight variations in the amount of current.

One experiment is typical of all, and is illustrated in the following table:

Treatment of 84 *gallons of milk for infant use.*

Time	Terminal voltage	Amperes	Terminal Temperature ° C.
8.47	3950	2·3	61·5
9.0	3950	2·25	60
9.10	3900	2·2	60·5
10.0	3900	2·22	63
10.15	3975	2·15	62·5
10.30	4050	2·2	63
10.40	4050	2·15	63
11.0	4150	2·1	63·5
11·20	4200	2·02	62
11.35	4200	2·05	64
Noon	3650	2·19	62

This milk was used for feeding infants, and proved very satisfactory. Neither by chemical examination nor by experiment on animals were we able to detect any decomposition or other chemical or physical changes in the milk.

In these communications we claimed that the electrical current had a direct killing action on the bacteria contained in milk, but recognised that the temperature necessarily produced during the process of sterilisation played some part, though in our opinion, a minor one. At the request of the Medical Research Council Sir Oliver Lodge and Prof. Leith repeated our experiments at Birmingham, and obtained similar results to those obtained by us. They, however, maintained that the destruction of the bacteria was a thermal effect. After an examination of the Report made by Lodge and Leith, the Medical Research Council stated that "In the opinion of the Council the experiments in Birmingham, though they entirely support the practical results obtained by Prof. Beattie and Mr Lewis at Liverpool, were not complete enough on their bacteriological side to settle, finally, the question whether the electrical current has a direct bactericidal effect or whether it acts purely as a thermal agent."

We now present further evidence in support of our claim that the electrical current is the important bactericidal factor. It is admitted that milk and other fluids can be sterilised by heat generated by electric currents, and we showed that a considerable degree of sterility could be obtained by means of direct

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currents, especially in the neighbourhood of one of the electrodes. This, we believe, was wholly due to the heat produced in that area. These experiments suggested the use of an alternating current, and eventually led to a modification of the apparatus, and to the regulation of the current, time of exposure, etc., in such a way as to reduce the temperature at the outlet and the time of exposure in the apparatus, to a point at which it would be almost impossible to regard the temperature as more than a minor factor in the killing of the bacteria.

It was found, early in these investigations, that a high degree of destruction of bacteria took place, if the conditions of the experiment were such as to produce a temperature at the outlet of the lethal tube of 70° C., but, if the conditions were varied, the same, or even a greater degree, of destruction was obtained with a lower outlet temperature—62° to 63° C.

Eventually, standard values for time of exposure, voltage, current density and temperature curve, were established.

By alteration of these "standards" the apparatus, which is described in a former paper (Beattie and Lewis, 1920), could be converted at will into a simple heat steriliser, or it could be shown to produce very little sterilising effect.

Various observers have attempted to use electricity as a direct sterilising agent for fluids, but the results have been, on the whole, unsatisfactory, and largely, we believe, because heat has been the principal factor in the process. To take one example only—the sterilisation of milk by the "Electro-Pure" process. In this process the milk is steam-heated to 40° C., it then passes through a series of open cascade cups in which there is an electrode. The milk leaves the machine at a temperature of "about 70° C." An alternating current of 2300 volts is used, but since "the alternate electrodes are opposite" it follows that the voltage-drop between two adjacent cups is of low value. In other words, the original high voltage is so split up that each cup receives only a particular fraction of the current. Under these circumstances, it is difficult to imagine other than a purely thermal effect. In investigating this process Anderson and Finklestein noted that gas-forming organisms (presumably *B. coli* group) were present in 0·1 c.c. of the milk when the outlet temperature was 68° C. in one experiment, and present in 0·001 c.c. with a similar outlet temperature in a second experiment. In a third experiment, a temperature of 71° C. showed similar organisms in 0·001 c.c.

They found that the milk passed through the machine in from five to thirty-four seconds, and experiments designed to show the effect of heat alone, in which milk was externally heated to 70° C. for ten to twenty seconds, gave even better results than this electrical method. They conclude that "it is the high temperature that so effectively destroys bacteria in milk, rather than the electric current itself." This conclusion is obviously justified on the experimental evidence. Our results were obtained with a lower temperature and with a time exposure about half as long.

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In an early series of experiments designed to test the reliability of our lethal tube, under standard conditions, it was found, on one occasion, that one of the electrodes had become disconnected. The result was, that the current passed through the outlet-half of the tube only, and therefore the milk received current for about one-half the normal time. The outlet temperature was 64° C., and yet plating showed 786 *B. coli* per c.c. of the milk. During the same series of experiments with the electrodes properly connected, the treated milk showed no *B. coli* per c.c. with an outlet temperature of 62° C., that is, at the higher temperature *B. coli* survived, at the lower temperature, on the same day and in the same sample of milk, they were killed.

It is true that in the interrupted experiment the current was acting for only one-half of its normal time, but as the normal time, *i.e.* for a rise from say 40° to 62° or 64° is only about 3 seconds, it is difficult to imagine that $1\frac{1}{2}$ seconds, at a temperature even of the maximum of 64°, could make any appreciable difference to the results.

Our conclusion from this experiment, is, that the current, as such, plays an essential part.

Further Experiments on this Point.

The normal speed at which milk flows through the lethal tube in the experimental apparatus used by us, under standard conditions, is 550 c.c. per minute; if, therefore, the speed be reduced, the electrical conditions must be altered to maintain the outlet temperature at its proper level. This can be brought about by a reduction of the voltage and amperage. The result is a reduction of the amount of current below that necessary for sterilisation, and a survival of the bacteria.

The experiments recorded in Table I illustrate this point.

Table I.

Speed in c.c. per minute	Outlet temperature	*B. coli* surviving	Electrical conditions
225	60° C.	8880	
—	61° C.	1740	
—	63° C.	79	Abnormal reduction of voltage and amperage
125	63° C.	784	
225	62° C.	578	
285	63° C.	252	
550	63° C.	Nil	Normal

From this it is apparent that when standard conditions are maintained *B. coli* are destroyed, but even if the outlet temperature is maintained at 63° C. any real variation from these conditions reduces the destructive power of the apparatus very considerably. It therefore seems to us impossible to maintain that the destruction is purely thermal.

Experiments with B. tuberculosis.

The general experience is, that a temperature of 60° C. for 20 minutes is the minimum thermal requirement to kill *B. tuberculosis*, and that occasionally a much higher temperature for varying times fails to destroy it.

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Thus, Rosenau (1908) concluded that an exposure to 60° C. for 20 minutes was necessary.

Orla-Jensen (1921), quoting Harding and Rogers, states, in speaking of Pasteurisation, "A far better result is obtained at 80° C. in a continuous pasteuriser (for two minutes), and as this temperature is necessary to ensure the destruction of the tubercle bacillus, it has been fixed as the minimum by the Danish Pasteurisation Law."

A. Besson (1913) states that exposure to 70° to 75° C. for ten minutes kills cultures of tubercle bacilli, and that in moist sputum the bacillus is not destroyed at 75° C., but is killed in five minutes at 100° C.

Hiss and Zinsser (1922) give the thermal death point of the bacillus in fluid media as 60° C. in 15 to 20 minutes, 80° C. in 5 minutes, 90° C. in 1 or 2 minutes.

Lane Claypon (1916), commenting on Rosenau's work, says: "It seems hardly possible to arrive at a definite temperature at which tubercle bacilli are killed (in milk), but it seems reasonable to suppose that a temperature of 60° C. for 20 minutes may be sufficient, or a short period at 95° to 100° C.

Rosenau, as quoted by Lane Claypon (1916), summarising other workers' experiments, gives results as follows:

Sormani	(1884)	T.B. not killed at	90° C.	for	10	minutes
Forster	(1892)	,, ,,	60° C.	,,	5	,,
De Man	(1893)	,, ,,	60° C.	,,	45	,,
Marshall	(1899)	,, ,,	60° C.	,,	10	,,
Morgenroth	(1900)	,, ,,	70° C.	,,	10	,,
,,	(1900)	,, ,,	100° C.	momentary		
Beck	(1900)	,, ,,	80° C.	for	3	minutes
Galtier	(1900)	,, ,,	85° C.	,,	6	,,
Herr	(1901)	,, ,,	80° C.	,,	5	seconds
Barthel and Stenström	(1901)	,, ,,	70° C.	,,	15	minutes
Bang	(1902)	,, ,,	60° C.	,,	15	,,
Rullmann	(1903)	,, ,,	60° C.	,,	20	,,
Zelenski	(1906)	,, ,,	76° C.	,,	20	,,

The Committee on Production and Distribution of Milk (1918), in its report on the Pasteurisation of Milk, Part I, quoting Delépine (Food Report, No. 21, Local Government Board) says that "Delépine found that a mixture of milk rich in tubercle bacilli, with 102 parts of non-tuberculous milk, heated to 77° C. for two minutes, was capable of producing tuberculosis on inoculation into guinea-pigs. On heating the same mixture of milk to 92° C. similar lesions were produced in guinea-pigs inoculated with it. It is questionable, therefore, whether any temperature short of boiling point can be relied upon to destroy the tubercle bacillus in milk."

A comparison of these results with those obtained by the electrical method is given in the following experiments.

A quantity of milk, rich in *B. tuberculosis*, obtained from a cow with advanced tuberculosis of the udder, was passed through the apparatus, and at intervals the electrical conditions were varied, samples obtained, and examinations made.

The following are the results:

(1) *Control Milk.* Four guinea-pigs were inoculated with the cream and deposit from 100 c.c. and all developed widespread tuberculosis.

(2) *Electrical conditions normal—outlet temperature* 63–64° *C.* Five four-ounce samples were collected, pooled, quantities were centrifuged, and the cream and deposit inoculated into two guinea-pigs. Each guinea-pig received cream and deposit representing 100 c.c. of the milk. The guinea-pigs, killed at the expiration of six weeks, showed no trace of tuberculosis.

(3) The quantity, and consequently the voltage of the current, was then

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slightly reduced, as indicated in a slight temperature fall. 100 c.c. samples were taken, and these, after centrifuging, were inoculated into guinea-pigs.

(*a*) At 60° C. guinea-pig No. 1 showed moderate infection, guinea-pig No. 2 showed very slight infection.

(*b*) At 60·7° C. to 62° C. both guinea-pigs showed moderate infection.

(*c*) At 60° C. to 61° C. guinea-pigs showed extensive infection.

(*d*) At 62° C. guinea-pigs showed no trace of infection.

(4) The electrical conditions were then re-adjusted to normal; the temperature rose to 63°–64° C. as before, and the guinea-pigs, after inoculation, showed, as in the first series, no trace of infection.

Here, then, we have a series of experiments, varying in the electrical conditions, with constant flow, and a total temperature excursion of only 4° C., but with results widely different.

It will be observed that the temperature in Experiment (3) was always lower than in (2) and (4); further, that in (3*d*) sterilisation was effected. It might, therefore, be maintained that the death point of the bacilli was from 62° C. to 64° C., and that the results obtained in Exp. 3 were not due to the alteration in the electrical conditions, but to the temperature being below the death point. If only a few bacilli remained alive infection would take place.

In answer to such criticism, we point out that the temperature only exerts its influence for a few seconds (the results are the same if the milk is cooled immediately it escapes from the outlet tube), and, if the results are produced by heat alone, then we must admit that exposure to a temperature of 62°, 63° or 64° C. for a few seconds is adequate for the destruction of large numbers of *B. tuberculosis* in milk. As we have pointed out, this is contrary to our experience, and to the observations of a large number of workers on this subject.

Taking our experiments on *B. coli* and on *B. tuberculosis* in the light of the observations referred to above, it seems to us a reasonable conclusion that, in the lethal tube, it is the electric current itself that is the primary lethal factor, and that the heat produced is merely an adjuvant.

Experiments with Direct Heat, under Conditions similar to those in the Electrical Tube.

In experimenting with direct heat, difficulty was experienced in obtaining conditions which accurately approached the thermal factors in the lethal tube and we realised that results, to be really comparable, must be obtained in apparatus which reflects those conditions. Thus the milk must be in flow, the temperature rise must be from room temperature to 42° C. in from 7 to 9 seconds, and from 42° C. to the maximum of 62° to 64° C. (usually 62°) in a further 3 to 5 seconds. In other words, the temperature must increase from 42° to 64° C. in a total period of, approximately, 14 seconds, and for not more than 4 seconds of this period must the temperature be higher than 55° C. Such conditions are probably impossible to reproduce by externally applied heat.

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Again, in the electrical lethal tube the heat is generated internally, that is, a given organism carrying its share of current (which it must do if it be in a field of a potential higher than is demanded by its own specific resistance) will become hot by reason of heat produced within its own substance. We cannot reproduce this internal heat, but we think it may be assumed that it is more likely to prove fatal, within limits, than the same degree of heat applied externally.

It has been suggested that the organisms may be killed as the milk passes over the hot electrodes. It can, however, be shown that the electrodes themselves do not generate any appreciable degree of heat, but become hot because of heat absorption from the warm milk. The material of which the electrodes are composed is stout copper, and it is a matter of common knowledge that such electrodes could carry enormously heavier currents of electricity without becoming hot, than they are ever called upon to carry when functioning in the lethal tube. But to demonstrate that the electrodes do not heat the milk, the following experiments were made:

(*a*) Additional electrodes were interposed in the external circuit of the apparatus, so that all the current used in the apparatus passed through them before reaching the lethal tube. Fats of low melting point were placed on to these electrodes, and the full current switched on, as milk was passing through the machine. The fats did not melt, although their melting points were much lower than the temperature of the issuing milk.

(*b*) Similar fats were placed on the functioning electrodes, milk was passed through the apparatus, and the full current applied. Here again, on the inlet electrode, the fats remained solid, showing no heat production there. At the intermediate "positive" electrode, the fats gradually softened, and at the outlet electrode ("negative" and in parallel with the inlet electrode) the softening was more marked and rapid, thus showing that these electrodes became gradually warm as they were washed by the onflowing milk.

It seems, therefore, quite clear that the electrodes are not the source of heat, but are themselves warmed by the heat generated in the milk.

Experiments in which milk, or infected water, was passed through a long steel tube, heated externally by serial Bunsen flames, were made, but were not continued because it was felt that the intense local heat necessary to ensure an average outlet temperature of 62° to 64° C. was largely responsible for any sterilisation; that the outlet temperature reading did not accurately show the heat conditions of the tube, and that, consequently, the results would not be comparable with the results obtained in the electric tube. A form of apparatus which was finally decided upon consisted of a glass tube, similar in size to that used in the electrical apparatus. This was supported in a horizontal position and T-pieces placed at each end; a U-tube to hold a thermometer was attached to the outlet end, and an aspirator containing the bacterial suspension to the other. A resistance coil was then threaded through the T-pieces and connected with a source of direct current. This is shown in Fig. 2.

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The suspension was allowed to flow through the apparatus and the direct current from the ordinary lighting circuit turned on. The passage of the electricity of course heated the coil, which heat was immediately transferred to the moving column of liquid. The amount of current used was regulated to give various outlet temperatures, and the coils of the wire acted as a fairly

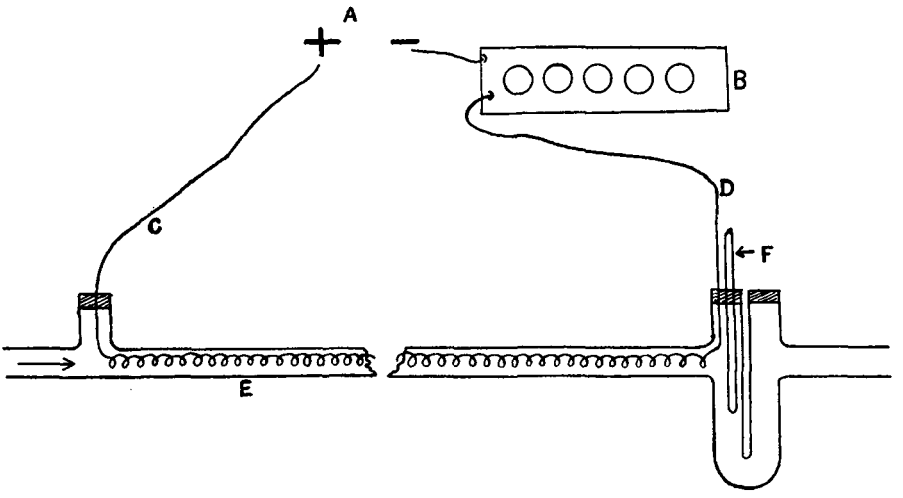

Fig. 2. *A*, wall plug; *B*, lamp resistance; *C* and *D*, electrical leads; *E*, Heating coil; *F*, thermometer. ⟶ direction of flow.

efficient mixer. Thus, at a given rate of flow, the conditions approximated somewhat to those of the "lethal tube." The rise of temperature was similar, and the heat equally distributed. By such distribution of the heat, the intense local heat of external application was avoided, and the outlet temperature was a guide to the maximum temperature generated in the tube.

We record the following representative experiments with this apparatus.

(*a*) Water with *B. coli* added was passed through the tube at a speed corresponding to 30 seconds' exposure. Samples were collected at various temperatures, cooled and immediately plated, as under.

	Control	
Outlet temperature at	62° C.	All plates showed innumerable colonies of *B. coli*, with no apparent difference between the control and those subjected to heat
,, ,,	,, 65° C.	
,, ,,	,, 75° C.	
,, ,,	,, 80° C.	

(*b*) Speed corresponding to 2·5 minutes, other details as above. Outlet temperature 62° C.

Result. No apparent sterilising action—numerous *B. coli* present.

It would thus appear that a temperature of 80° C. acting for a brief period only is here unable to sterilise a fluid containing *B. coli*; whereas in the high-tension alternating electrical apparatus *B. coli* are killed at a much lower temperature, and with a shorter time exposure.

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(*c*) Ordinary water was slightly infected with *B. coli* and passed through the electrically heated coil tube at a speed which, by calculation, gave an exposure to the highest temperature for 4–5 seconds. Samples were taken at different temperatures and plated as below.

Table II.

	B. coli per c.c.
Control	696
At 62° C.	732
„ 65° C.	578
„ 67° C.	594
„ 68° C.	492
„ 70° C.	276

Further Experiments with Direct Heat.

A beaker of ordinary milk, containing *B. coli*, was placed in a water-bath at 64° C., and the milk constantly stirred. In 2·5 minutes the temperature of the milk rose to 62° C., and then remained constant at 62° to 63° C. Twelve samples of the milk were then pipetted and inoculated into Bile Salt Lactose Litmus broth, with time intervals of 0·25 minute. The minimum time of exposure was thus 0·25, and the maximum 3 minutes. The result of incubation showed that living *B. coli* were present in each of the tubes.

Thus, exposure to 62° to 63° C. for 3 minutes failed to destroy the *B. coli* present in the milk, at which temperature, for a few seconds, the electrical machine invariably is able to do.

This type of experiment was repeated, using sterile milk with *B. coli* added; the temperature was 62° to 65° C. Sixteen tubes of Bile Salt media were inoculated at intervals of 10 seconds, and incubated. In this experiment the shortest time of exposure was 10 seconds, and the largest $1\frac{2}{3}$ minutes.

All the tubes developed acid and gas, which on plating yielded cultures of *B. coli*. A further experiment was as under:

One c.c. of milk was added to each of a series of Bile Salt tubes, which were then placed in a water-bath at 62° to 68° C. The temperature of the tube contents rose from 62° to 65° C. in one minute. The tubes were then left in the bath for further periods, cooled and incubated. The result is shown in the table.

Table III.

Time	Temperature	Result
10 seconds 20 „ 30 „ 40 „ 50 „ 60 „ 80 „ 100 „	62°–65° C.	All developed *B. coli*

Two further tubes, in which the temperature rose to 67° C. remained in the bath for 110 and 120 seconds respectively. In these, after incubation, there was no growth of *B. coli*. Thus, in this experiment 100 seconds at 62°

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to 65° C. failed to kill *B. coli*, but the organisms were destroyed in 110 seconds at 62° to 67° C. A further modification of the experiment was made.

Sterile bile salt broth tubes were placed in a water-bath at 65° C., and allowed to remain there for 5 minutes in order that the internal temperature of the tube should be equalised. 1 c.c. of coli-containing milk was then added to each tube, and these were taken out and cooled at time intervals as follows:

0·5 minutes 1·0 „ 1·5 „ 2·0 „ 2·5 „ 3·0 „ 3·5 „	All these showed a growth of proved *B. coli*
4·0 „ 4·5 „ 5·0 „	These showed no *B. coli*, but other organisms were present

An experiment with milk in capillary tubes was made as follows:

Milk was distributed in 0·05 c.c. quantities in sterilised capillary tubes, sealed, and heated in water at 62° to 63° C. for varying periods. The results were:

Control	Innumerable bacteria per c.c.
10 seconds 13 „ 18 „	„ „ „
25 „	149,200 bacteria per c.c.
30 „	63,600 „ „
45 „	79,400 „ „

The experiments recorded, although made under heat-conditions approaching those occurring in the electrical lethal tube, differ, as we have already stated, in certain important points, and comparison is difficult, but our experiments, we think, give every advantage to the direct heat method, and yet it has failed to produce such successful results as the electrical process.

Discussion of the Various Factors in the Experiments.

In any experiment in which a volume of cool fluid is heated by means of immersion in a water-bath, time is taken for the fluid to reach its maximum temperature, and the effect of this must be considered in relation to the final result. This factor is, however, largely discounted in the experiments recorded above, in which relatively small volumes of milk were added to relatively large volumes of hot fluid media, and also in the experiments where the milk to be treated is enclosed in thin-walled capillary tubes. In both types of experiment the milk is very rapidly heated to its maximum temperature, and therefore they are fairly comparable with the rapidity of rise in the electrical apparatus.

In Relation to Externally Applied Heat.

When a cool fluid is passing through a hot tube, it is obvious that the tube itself must be hot enough to counteract the cooling effect of the fluid if a given temperature is to be maintained. The tube, therefore, must be hotter than the

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average temperature of the issuing fluid. The destruction of bacteria during such treatment consequently depends in part upon the involvement of the bacterium in zones of relatively intense heat. Again, water (or milk) is a relatively bad conductor of heat, and because of the varying densities due to the heat, the fluid is to a certain extent passing through the tube in telescopic fashion, cylindrical streams, as it were, varying in velocity, the innermost one being the coolest, and the outermost the hottest. On reflection it is obvious that these conditions will markedly influence the end result of the experiment.

Sterilisation of a moving column of fluid can be readily effected by passage through a hot tube, but to compare such degree of sterility with that occurring in the electrical lethal tube is fallacious unless both time and maximum temperature be taken into consideration. If a tube 55 inches long, and $\frac{3}{8}$ inch in diameter be suspended in a water-bath, and milk and water allowed to pass through, it will be found necessary to heat the surrounding water to 20–25° C. higher than a given issuing temperature with a delivery of 500 c.c. per minute. Now the electrical tube delivers milk at a maximum temperature of 62° to 64° C. at the rate of 550 c.c. per minute, and to maintain this temperature and speed with externally applied heat, it was found that *a temperature of* 88° *C. was necessary in the surrounding water.* It follows, therefore, that the outlet temperature is not a guide to the maximum temperature reached in portions of the milk during the journey through the tube, and the bacteria are subjected to a high temperature; it may be over 80° C. at the periphery of the milk, and therefore the sterility is no index that the bacteria were killed at the outlet temperature, *i.e.* the temperature of a mixture of overheated and underheated milk.

If the delivery from the tube be reduced to 100 c.c. per minute, a bath temperature of about 70° C. will maintain an outlet temperature of 64° C., but in this case the organisms are under treatment for a period of time five times as great as in the electrical tube.

In all our experiments in which the heat conditions most nearly approximated to those of the electrical tube, the test organisms were not killed, even when the temperature was higher, or when the period of treatment was longer than in the electrical process. Thus an outlet temperature of 80° C. for a few seconds failed to kill *B. coli*, and 62° C. for a 2·5 minute passage failed also to kill.

Again, temperatures of 68° and 70° C. only brought about partial sterilisation with a time factor corresponding to the electrical method.

In another experiment 62° to 63° C. failed to kill *B. coli* in 3 minutes.

In another, *B. coli* were alive at the expiration of 3·5 minutes, but dead with 4 minutes' exposure.

Again, with milk enclosed in capillary tubes, *B. coli* and other organisms were still alive after 45 seconds' exposure.

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Series of Experiments with the Electrical Apparatus.

The object of this series of experiments is to show that a considerable degree of sterilisation can take place in the electrical apparatus at a temperature, which, of itself, is admittedly not capable of killing bacteria—the time factor in each case being equal.

Exp. 1. A heavily infected suspension of *B. coli* in water was adjusted by the addition of saline to a point such that it required approximately the same voltage as milk in order to pass the requisite quantity of electricity. This suspension was passed through the apparatus at the normal speed, and by suitable slight alterations in the amount of current, variations in temperature were obtained. The fluid was first of all passed through the lethal tube under standard conditions, and samples taken for plating; the conditions were then varied, and other samples collected and similarly plated. The results are set out in the following tables.

Table IV.

Type of samples	*B. coli* per c.c.	Percentage reduction
Control (untreated)	2,400,000	—
At 61° C.	Nil	100
„ 59° C.	Nil	100
„ 57° C.	27	99·9
„ 56° C.	480	—
„ 55° C.	31,900	—
„ 54° C.	52,200	98

A portion of the same suspension, used in this experiment, was heated in small test-tubes to 54° C. for a few seconds, and then plated. The plates were too crowded to count, and showed no obvious difference from the control specimen.

Table V.

Type of samples	*B. coli* per c.c.	Percentage reduction
Control	351,200	—
Treated at 61° C.	Nil	100
„ 59° C.	496	99
„ 57° C.	13,440	96
„ 55° C.	No appreciable variation from control specimen	
„ 54° C.		

Exp. 2. A portion of the same suspension (Table IV) was treated at 63° C. (*i.e.* full ordinary temperature), but only two electrodes instead of three were used, thus the organisms were only under treatment for about half the usual time. Plating out showed that under these circumstances a few of the *B. coli* survived, viz.:

(*a*) 9
(*b*) 15 } *B. coli* per c.c.
(*c*) 3

Exp. 3. In this experiment, in which ordinary milk was used, the velocity was normal for the first part, and increased by 25 per cent. for the second part,

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and during each part of the experiment the amount of current used was altered, giving slight variations in temperature. The results are given in Table VI.

Table VI.

Temperature	Ordinary speed	25 % increase in speed
63° C.	*B. coli* per c.c. 0	1
62° C.	„ „ 0	17
60° C.	„ „ 0	24
59° C.	„ „ 2	183
62° C.	„ „ 0	10
62°–63° C.	„ „ 0	33
61° C.	„ „ 0	19

This shows that at normal speed *B. coli* are killed, even when the temperature is subject to certain variations, but when the speed is increased *B. coli* survive, even at 62°–63° C. The decreased time of exposure of the milk to the current at the critical temperature is only a fraction of a second.

Exp. 4. Milk was subjected to normal treatment (Sample No. 1), then slight variations were made in the quantity of current, to give the required temperature; the speed of flow remained constant. At intervals samples were plated. The results are shown in Table VII and a similar experiment is given in Table VIII.

Table VII.

Sample No.	Temperature	*B. coli* per c.c.	Percentage reduction
Control	—	3120	—
1	63° C.	Nil	100
2	60° C.	3	—
3	52° C.	59	—
4	55° C.	102	—
5	55° C.	72	—
6	54° C.	126	—
7	52° C.	61	—
8	62° C.	Nil	100
9	57° C.	Nil	100
10	53° C.	67	—
11	48° C.	970	69

Table VIII.

Sample No.	Temperature	*B. coli* per c.c.	Percentage reduction
Control	—	2440	—
1	62° C.	Nil	—
2	59° C.	Nil	—
3	58° C.	21	—
4	55° C.	63	—
5	49° C.	504	80
6	57° C.	8	—

This series of experiments (Nos. 1 to 4) show that *B. coli* can be destroyed below their recognised thermal death point. Thus, in Table IV there is complete destruction of *B. coli* at a temperature of 61° C. for a few seconds; 99·9 per cent. reduction at 59° C., and 98 per cent. reduction at 54° C.

In Table V there is complete destruction at 61° C.; 99 per cent. reduction at 59° C., and 96 per cent. reduction at 57° C.

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Table VII shows a similar reduction in the total number of *B. coli* surviving, and also that as the electrical conditions vary, so does also the efficiency of the sterilisation. Under normal conditions all *B. coli* were killed, but as the quantity of current was gradually diminished, some of the *B. coli* lived; as the current was re-established to the ordinary level, complete sterility was again obtained.

In one sample at 57° C. no *B. coli* were found, and at a temperature of 48° C., 69 per cent. of the organisms were destroyed. In sample 5, Table VIII, 80 per cent. of the total *B. coli* were destroyed at a temperature of 49° C.

These facts again make it difficult to deny the direct lethal action of the current.

Table VI lends additional evidence. Under normal conditions complete destruction of *B. coli* was obtained, but at a corresponding temperature with a decreased period of exposure to the current, a certain number of organisms survived.

It has been suggested that a simple way of even more definitely proving our contention would be to cool the milk as it passes through the lethal tube, thus, it is said, the final high temperature could be avoided. We do not agree. In the case of a metal conductor, artificial cooling would render it less resistant to an electric current, but in the case of a fluid conductor the reverse occurs. Thus, if the milk be artificially cooled, either before or during treatment, its electrical resistance would be increased, and a higher potential would be required in order to pass the necessary amount of current through it. The net result, therefore, would merely be to use more electricity, and the outlet temperature would remain the same. If the same voltage be used, the necessary amount of current would fail to pass and sterilisation would not occur.

General Conclusions.

(1) That when milk is allowed to flow through an externally heated tube, destruction of the bacteria may take place, but the temperature of the issuing milk is not an accurate indication of the maximum temperature actually employed. Partial or even complete sterilisation may occur owing to the organisms passing through zones of a much higher temperature than that of the outlet.

(2) That in apparatus designed to minimise these hot zones, sterilisation does not take place in the time, and with the degree of heat, which is effective in the electrical lethal tube.

(3) That in the electrical lethal tube, bacteria can be destroyed at a temperature much below their thermal death point.

(4) That the efficiency of the electrical apparatus is dependent on the high tension current used, as such, in combination with the correct time of exposure to its action.

(5) That all the experiments recorded point to the fact that heat is not the

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main factor in the destruction of the bacteria in this process, and indicate that the electric current, so used, is an important destroying agent.

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