THE VITAMIN C CONTENT OF ELECTRICALLY TREATED MILK.

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(With Plate VII and Graphs 1–12.)

While a sufficient supply of Pure, Grade A, tuberculin-tested milk has not yet been attained and most consumers have to be contented with milk that has been pasteurised or otherwise rendered innocuous, improvements in the technical treatment, which may preserve the original nutritive elements, are very welcome. In a former paper[1] I drew attention to the fact that the small vitamin C content in pasteurised milk is due to the air (oxygen) in the milk. In this paper I shall discuss a new method of pasteurisation, which should find its way into practice because of its advantages over that usually employed. The method is that examined and recommended for its favourable results by Beattie and Lewis at Liverpool, Leith at Birmingham, and Sir Oliver Lodge.

I do not intend to consider electrical sterilisation from a bacteriological standpoint, but refer the reader to the publications by Beattie and Lewis (1920, 1925)[2].

What interested me in particular was whether the vitamins could resist a current of some thousand volts, as Beattie and others have applied. This could only be determined by feeding experiments. Beattie and Lewis refer to "feeding experiments" it is true, but they restrict themselves to the following statement:

Young kittens were fed on electrically-treated milk for several weeks. The controls fed on raw milk all died, whilst those fed on the treated milk regularly increased in weight. We realise that such experiments, to be of any value, must be carried out over a very long period, and circumstances prevented us from continuing this work. For several months babies were fed on this milk, and, so far as we could ascertain, did extremely well. Two of these were under close observation the whole time, but with the great majority of the cases the observations were very erratic, and no conclusions of any value could be drawn from them. The two kept under close observation were the children of medical men, and it can merely be said that both parents expressed their greatest satisfaction with the milk as a food. But we fully agree that the whole of the evidence on the food value is too feeble to enable us to draw conclusions. Possibly quite as good results would have been obtained by ordinarily heat-sterilised milk. There is no doubt, however, that children take the milk very readily, even those who object to heat-sterilised milk.

[1] van Leersum (1925). *Nederl. Tijdschr. v. Geneesk.* Part 1, p. 338.

[2] Beattie and Lewis (1920). On the destruction of Bacteria in Milk by Electricity. *Med. Res. Council Special Report Series,* No. 49 (London). Beattie and Lewis (1925). The Electric Current (apart from the heat generated). A bacteriological agent in the sterilisation of Milk and other fluids. *J. of Hygiene,* xxiv. 123–137.

https://doi.org/10.1017/S0022172400017575 Published online by Cambridge University Press

Obviously no sufficient information is here given concerning the resistance of vitamins to the action of the electric current, nor could Professor Beattie supply any when, about two years ago, I had the pleasure of discussing this matter with him. Therefore I resolved to make experiments, perforce confined for the present to the antiscorbutic factor, the so-called vitamin C.

The apparatus I used was similar to that Professor Beattie showed me in his laboratory and which he has described and figured; hence I need but refer to a few details. It was capable of sterilising 250 c.c. of milk in a minute. For the supply I used a Mariotte bottle, which enabled me to maintain the rate of flow needed for the desired action of the electric current. In test experiments the temperature of the outflowing milk was 65–70° C., later I succeeded in keeping the temperature constant at 64–65° C. The amperage of the current was ± 0·5, the voltage ± 2000.

The electrodes consisted of spiral copper-plates, measuring about 0·5 cm., and large enough to fill the electrode-chambers so that sufficient contact was ensured with the milk that flowed past.

Besides these copper electrodes, used by Beattie, I, for reasons which will be mentioned later, also used carbon electrodes of such a circumference as to almost completely fill the chambers, so that good contact with the milk was obtained.

For the investigation of the influence of the electric current of high tension on the vitamin C, guinea-pigs were used, because of their well-known susceptibility to scurvy and their never failing to get this disease within four weeks when put on a so-called scurvy-promoting diet. The ration I used consisted of oats 69 parts, autoclaved hay 25 parts, and kitchen-salt 1 part. During the experiment the animals were weighed twice a week. Those which had not died of scurvy or some attendant disease, were killed for reasons of economy after three months. All the animals, those that had been killed as well as those that had died, were autopsied and of each animal some ribs, the tibiae and the femurs, the bladder and the adrenals were examined microscopically. For the clinical and microscopical diagnosis I adhered to the descriptions of the changes in the tissues given by Holst and Froelich (1907)[1], Delf and Toser (1918)[2] and McGarrison (1921)[3].

I tried the electric pasteurisation on two kinds of milk, namely:

1. *Milk A*, a raw and quite fresh, pure, grade A, tuberculin-tested milk from the model farm, Oud-Bussum, which is situated at about 25 kilometres from Amsterdam. This sort of milk proved to be completely up to the mark, also as regards the content of vitamins. It is destined to be drunk raw without any danger to health.

2. *Milk B*, a raw, at least 16 hours' old milk of middling quality, as it is sold at the door in Amsterdam. This milk did not come up to a high mark;

[1] Holst and Froelich (1907). *J. of Hygiene*, VII. 634.
[2] Delf and Toser (1918). *Biochem. Journ.* XII. 416.
[3] McGarrison (1921). *Studies in Deficiency Disease.* Oxford Medical Publications. London.

especially as far as the content of vitamin C was concerned it left much to be desired, as I had previously found.

3. *Milks B* 1 and *B* 3, being milk B to which I added neutralised lemon juice in quantities of 1 and 3 per cent. respectively to raise the vitamin C content to a proper level.

The experiments were made in summer, when the milk cows were in the fields and had plenty of grass to eat. Therefore the difference in the content of vitamin of milk B was not owing to the food, but to the long exposure of the milk to the air (oxygen)[1].

EXPERIMENTAL.

1. *Provisional experiments with various quantities of milk A, to define the quantity needed to prevent scurvy in guinea-pigs on scurvy-producing diet.* (See Graphs.)

Two animals which had daily had 50 c.c. autoclaved milk died within 25 days of scurvy, as was to be expected.

Five animals fed daily on 25 c.c. milk A mixed with 25 c.c. autoclaved milk, died too, though not so soon. One of these animals, No. 129, survived for 70 days. All suffered from scurvy, as was proved by clinical observation and microscopic examination.

The 6 animals of the third group received 50 c.c. of milk A daily. Nos. 69, 132 and 134 were killed after 147, 141 and 137 days respectively, because they showed no signs of illness. Their tissues showed no pathological changes. Nos. 90, 93 and 133 died after a longer or shorter period, No. 93 did not die of scurvy. Nos. 90 and 133 suffered from an extravasation of blood in one knee-joint, but no other scorbutic changes were found.

Of the 3 animals of the fourth group which had received 75 c.c. of milk A daily, No. 136 died very early of an accidental disease. No. 137 survived 100 days and on investigation proved not to have had scurvy. No. 135 continued in good health and was killed after 136 days.

From these experiments it appeared that in most cases 50 c.c. of milk A were sufficient, but in some cases it was insufficient in the long run. Occasionally it appeared that this quantity did not remain sufficient while the weight of the body increased. Therefore I resolved to give the animals quantities in proportion to their weight, *i.e.* 30 c.c. of the electrically treated milk per 100 gm. of body-weight. Many control experiments have proved this quantity to be sufficient to protect guinea-pigs against scurvy when fed on scurvy-promoting diet.

2. *Feeding experiments with electrically treated milk A.* (See Graph.)

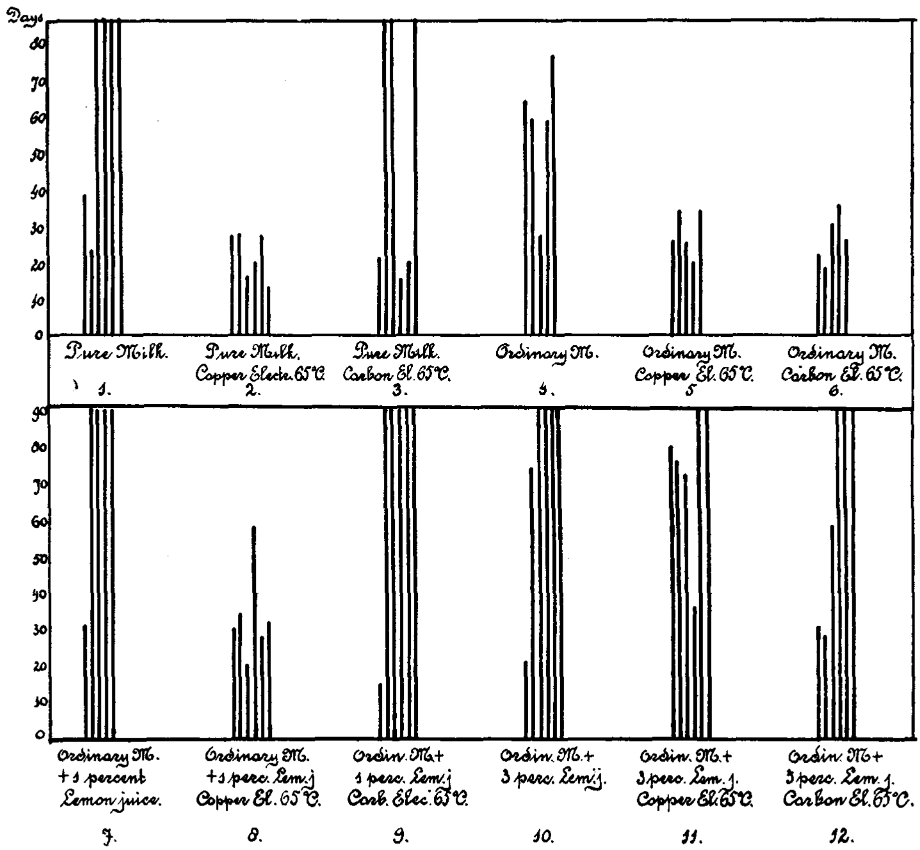
For this experiment copper electrodes were used.

Six guinea-pigs were used, 2 of which received 25 c.c. of electrically treated milk A (plus 25 c.c. autoclaved milk) daily; the other 4 received 50 c.c.

[1] van Leersum (1925). *Loc. cit.*

None of the animals of the two groups lived longer than 4 weeks. All died with signs of acute scurvy.

As in this series of experiments the temperature of the milk had occasionally risen to 70° C., therefore a few degrees higher than Beattie and Lewis allowed, I repeated this experiment with milk A heated to exactly 65° C., but the same results followed. (See Graph, 2.) They show that in milk of excellent quality the vitamin C is completely destroyed when the milk is electrically treated (with copper electrodes), for the animals fed on such milk did not sur-



Graphs 1–12. Giving a comparative view of the results of the experiments. The vertical lines represent the longevity of the guinea-pigs in days. Where a vertical line reaches the upper horizontal line, it means that the animal was finally killed, after the fixed period of three months.

vive those which had autoclaved milk in which there was no vitamin C, while the changes caused by scurvy in the tissues were equally extensive and intensive.

In connection with this we may refer to the experiments by Hess and Weinstock (1924)[1] on the catalytic action of minute amounts of copper in the destruction of antiscorbutic vitamin in milk. Their experiments leave no

[1] Hess and Weinstock (1924). *J. Am. Med. Ass.* LXXXII. 952.

doubt about the destructive action. Therefore Hess has warned against the use of copper vessels and tubes in milk plants.

3. *Feeding experiments with electrically treated milk A, with the use of carbon electrodes.* (See Graph.)

What led to the substitution of carbon electrodes for copper electrodes was the fact that, after electrical treatment, the milk proved to contain more copper than there had originally been in it. It is well known that milk always contains some copper in an organic, not active form, probably in combination with protein. Applying the aethyl-xanthate method recommended by Supplee and Bellis (1922)[1], Miss Honig, working in our laboratory, found the smallest quantity in milk A, before electrical treatment, 0·5 mg. per litre and after 0·7 mg. per litre; and in milk B 0·22–0·5 before electrical treatment and after 1–1·3 mg. to a litre. The assumption seemed justified that the copper which becomes detached from the electrodes possesses active properties and, as a catalytic agent, promotes the oxidation of vitamin C. Very small quantities suffice for catalytic action. Moreover, air (oxygen) is always present, for when milk is handled, when it falls into the pail, is driven to the factory, etc., it has many opportunities to become saturated with it. Kohman (1923)[2] found milk delivered at the factory for pasteurisation to contain 18 c.c. of gas per litre, 17·6 per cent. of which consisted of oxygen. In the same milk after pasteurisation, he still found 12·9 c.c., of which 15·5 per cent. was oxygen. The results of determinations made in our laboratory agree with this. Miss Honig found in three samples of milk B, at a temperature of 20° C., 1·41, 2·14 and 1·94 c.c. of gas (0° and 760 mm.) per 100 c.c. I found that the proportion of oxygen in these gas mixtures was much higher than Kohman states, namely in one case 23·1 vol. per cent. of oxygen to 76·9 vol. per cent. of nitrogen; in another case 27·4 vol. per cent. of oxygen to 72·6 vol. per cent. of nitrogen. It was to be expected that the composition of the gas mixtures found in milk would not agree with that of the atmospheric air, the power of absorption of water with regard to various gases being unequal. Winkler[3] found 18·68 c.c. of gas in 1000 c.c. water which had been saturated at 20° C. with air not containing carbonic acid and ammonia. The gas when measured consisted of 6·36 c.c. oxygen and 12·32 c.c. nitrogen, at 0° C. and 760 mm. Hg. So the proportion of oxygen was about 34 per cent.

It goes without saying that the temperature of the milk, 65° C., hastened the process of oxidation of the vitamin C. It played a less important part, however, as the oxidation also takes place, as appeared from former experiments, at 0° C. If carefully shut off from oxygen, vitamin C does not run any risk, however, even if the milk is more strongly heated than is necessary for ordinary pasteurisation.

[1] Supplee and Bellis (1922). *J. of Dairy Sci.* v. 455.

[2] Kohman (1923). *Industr. and Engin. Chem.* xv. 237.

[3] Landolt-Bornstein. *Physik. Chem. Tab.* 1923, I. 766.

Six guinea-pigs were used for a feeding experiment with milk A sterilised with carbon electrodes, while the same measures were taken as for former experiments. Three died early, showing no other change than an extravasation of blood at one of the knees. On careful examination no other scorbutic changes were found, so that the disease was not serious enough to account for their early death. This must have been due to some accidental ailment, the nature of which could not be ascertained.

The remaining 3 animals of this group continued in good health and were killed at the end of 3 months. They showed no signs whatever of scurvy.

It appears from this experiment that the vitamin C content greatly benefited by the substitution of carbon electrodes for copper ones. Yet it does not seem to have remained entirely intact, for, apart from the three cases which had a lethal course, the growth of the guinea-pigs Nos. 168 and 169 also left something to be desired. In the discussion of the next experiment I shall return to the problem of how the decrease of the vitamin C content is to be explained.

4. *Feeding experiments with milk B, raw, or electrically treated, with copper electrodes or with carbon electrodes.* (See Graph.)

As appeared from an experiment on 5 guinea-pigs and also on other occasions, this milk B, in quantities of 30 c.c. per 100 gm. body-weight, was insufficient to prevent scurvy in animals on scurvy-promoting diet. In the experiment on 5 guinea-pigs, the animals lived 27, 58, 59, 64 and 76 days respectively, and all suffered from subacute scurvy.

The results of the experiments with this milk, electrically treated, were very bad. There was no difference to be noticed between the vitamin C content of milk B treated with carbon electrodes and of that treated with copper electrodes. The 5 animals of the carbon electrode group did not live a day longer than those of the copper electrode group. All died of acute scurvy.

To explain this unfavourable result of the treatment with carbon electrodes, it must be remembered that the milk contained oxygen and that its temperature in the apparatus was 65° C. and probably higher close to the electrodes. Consequently, two important factors for the oxidation of vitamin C were also present in the case of the carbon electrodes, so it is not venturing too much to hold them responsible for the destruction of the vitamin which was never abundant in this kind of milk. They must have played a great part in producing the result.

As it proved too expensive in the long run to work with milk A, I used the cheaper milk B for the following tests, artificially raising the vitamin C content to a sufficient level by adding neutralised lemon juice.

5. *Feeding experiments with milk B to which, before electrical treatment, 1 per cent. neutralised lemon juice was added.* (See Graph.)

A control experiment on 5 guinea-pigs showed that 1 per cent. of lemon juice was sufficient to raise the vitamin C content of milk B to such an extent

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that this milk, in quantities of 30 c.c. per 100 gm. body-weight, could prevent scurvy (see Graph, No. 7).

Only one of the control animals died early, after 31 days. It had indeed a haemorrhage at one of the knees, but I did not find any other manifestation of scurvy. The other 4 were killed after 3 months, while in good health, and proved to be normal.

A second group of 6 guinea-pigs, Nos. 191, 192, 193, 194, 195 and 196, had milk B plus 1 per cent. of lemon juice, this mixture having been pasteurised with copper electrodes (see Graph, No. 8). The effect was striking: 4 animals died, within 5 weeks, of acute scurvy, only 1 lived for 56 days, but then also died of scurvy.

A third group, Nos. 185, 186, 187, 188, 189 and 190, was given the same mixture treated with carbon electrodes (see Graph, No. 9). The difference between the results obtained with this treatment and that with copper electrodes is a convincing argument for the vitamin-sparing effect of carbon electrodes, for, of the 6 animals of this group, 1 died after 15 days of an accidental disease, there being no question of scurvy; the other 5 were killed, still being in perfect health, at the end of the experiment.

6. *Repetition of the preceding experiment with milk B*, 3 *per cent. neutralised lemon juice having been added.*

Out of the 6 control animals, fed on the untreated mixture, 2 died after 21 and 74 days respectively. Neither suffered from scurvy, nor did any of the remaining 4, which were killed after 3 months. It is noteworthy that, notwithstanding the plentiful quantity of lemon juice, 2 guinea-pigs died before the time fixed had elapsed, which once more proves the well-known delicacy of the guinea-pig as a test animal.

Apparently the results of the feeding experiments with this mixture after treatment with copper and with carbon electrodes do not show any difference worth mentioning and it would seem that the substitution of carbon for copper was not, in this case of a milk of high vitamin C content, an improvement, for of the copper group 4 out of the 6 test animals died before the time set had elapsed and of the carbon group 3 out of 6 (see Graphs, Nos. 11 and 12). But the microscopical examination supplied the necessary light: whereas in the animals of the copper group, also in those killed after 3 months of apparently good health, microscopical scorbutic changes were manifest, there were none to be found in the animals of the carbon group. This essential difference does not become visible in the graph and this is a case in point to argue the necessity of careful and extensive microscopic examination of the tissues to support clinical observation and weighing in investigations of this kind.

It having appeared that the use of carbon electrodes spared vitamin C, it remained to be determined whether the use of carbon electrodes had an equally strong bactericidal effect as the copper ones. If it should appear that this was not the case, then the use of carbon could certainly not be called an improvement, for the benefit of preserving the vitamin in milk does not counterbalance the danger that arises from insufficienly sterilised milk. Beattie and Lewis have proved that, as far as sterilisation goes, the electrical method deserves full confidence. In their opinion milk can be rendered free from *B. coli* and *B. tuberculosis* by this method, at a temperature not higher than 63° or 64° C., and though it does not completely sterilise milk (for that matter neither does ordinary pasteurisation accomplish this) yet it reduces the number of bacteria by 99·93 per cent.

Beattie and Lewis used copper electrodes, however, and now the question arose if the carbon electrodes had an equally strong bactericidal action. To answer it, Professor van Loghem, Director of the Institute of Hygiene, University of Amsterdam, was kind enough to compare the content of bacteria in milk sterilised by means of copper electrodes to that in milk treated with carbon electrodes. The results of the examination will be found in the following tabular statements and in the report on milk infected with *Bacillus tuberculosis*.

Preliminary experiments with milk B proved that *B. tuberculosis* added thereto were infective for a guinea-pig when the bacilli were centrifuged out of the milk, suspended in saline solution, and injected into the animal. A similar suspension of the deposit obtained from milk B to which tubercle bacilli had not been added had no effect when injected into a second guinea-pig.

Test I with copper electrodes. On 15. vii. 1924 four loopsful of a suspension of bovine tubercle bacilli were added to 4 litres of raw milk B, and the milk was divided into two equal portions. Portion *A* was exposed to the effects of copper electrodes and portion *B* was not, so that it served as control: 500 c.c. of *A* and *B* were centrifuged and the corresponding deposits suspended in 4 c.c. of saline solution.

Test I. Two guinea-pigs received 1 c.c. of supension *A* each.

Result. In one animal some small glands palpable on 25. viii. Both animals were healthy on 18. ix. 24.

Control. One guinea-pig received 1 c.c. of suspension *B*.

Result. On 23. vii. glands enlarged, tubercular. On 25. viii. animal very ill with pus flowing from large abdominal swelling. On 18. ix. 24 animal killed. Inguinal glands on both sides much swollen and *B. tuberculosis* found; extensive tuberculosis.

Test II with carbon electrodes. On 27. iv. 1925, four loopsful of suspension of freshly isolated human tubercle bacilli in 3·5 c.c. saline were added to 2 litres of raw milk B and this was divided in equal portions *A* and *B*, each containing a half of the *B. tuberculosis* suspension, etc., as described under Test I (*vide supra*).

Test. Two guinea-pigs received 1 c.c. of suspension *A* each.

Result. One animal was killed on 8. vi. 25 and autopsied with negative result in respect to lesions and tubercle bacilli; emulsions of its spleen, liver

https://doi.org/10.1017/S0022172400017575 Published online by Cambridge University Press

ELECTRICAL STERILISATION OF MILK.

Record of bacterial colonies counted after 48–72 hours' cultivation. Liq. = liquefied; ∞ = innumerable colonies.

Table I. *Pure Milk A (fresh). Copper Electrodes. Highest temp.* 70° *C.*

Quantity of milk in c.c.	Raw								Pasteurised							
	Gelatin at 24° C. Experiment				Agar at 37° C. Experiment				Gelatin at 24° C. Experiment				Agar at 37° C. Experiment			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
	Colonies: 48 hrs.				Colonies: 72 hrs.				Colonies: 48 hrs.				Colonies: 72 hrs.			
0·5	—	—	—	—	—	—	—	—	±60	±200	0	4	±250	±200	25	20
0·1	±400	±300	Liq.	Liq.	135	112	±100	±350	35	64	0	4	40	65	5	16
0·01	±120	50	±900	Liq.	18	25	±250	25	2	5	0	0	1	2	1	2
0·001	10	4	65	±120	0	0	20	5	—	—	—	—	—	—	—	—
0·0001	4	1	10	18	0	0	5	6	—	—	—	—	—	—	—	—

Ordinary Milk B (not fresh). Copper Electrodes. Highest temp. 70° *C.*

	Experiment				Experiment			
	I	II	I	II	I	II	I	II
0·5	—	—	—	—	15	22	±300	±320
0·1	Liq.	Liq.	∞	∞	18	4	155	±160
0·01	Liq.	Liq.	∞	∞	4	1	7	2
0·001	∞ (part liq.)	∞	∞	±900	—	—	—	—
0·0001	∞ (6 col. liq.)	±300	±1440	±200	—	—	—	—

Table II. *Ordinary Milk B (not fresh). Carbon Electrodes. Highest temp.* 65° *C.*

Quantity of milk in c.c.	Raw				Pasteurised			
	Gelatin at 24° C. Experiment		Agar at 37° C. Experiment		Gelatin at 24° C. Experiment		Agar at 37° C. Experiment	
	I	II	I	II	I	II	I	II
	48 hrs.	72 hrs.	48 hrs.	72 hrs.	48 hrs.	72 hrs.	48 hrs.	72 hrs.
0·5	Liq.	Liq.	∞	∞	±150	Liq.	±9000	±9000
0·1	,,	,,	∞	∞	±125	,,	±7500	±8000
0·01	,,	,,	±3500	±4200	±160	±400	± 200	± 300
0·001	,,	,,	± 450	± 500	± 50	± 85	11	17
0·0001	110	150	28	35	15	60	2	2
0·5	Liq.	Liq.	∞	∞	±100 (?)	Liq.	±7000	±7000
0·1	,,	,,	∞	∞	±500	±800	±5800	±5000
0·01	,,	,,	±3800	±5000	±175	±400	± 150	± 200
0·001	,,	,,	± 300	± 300	± 30	±100	6	11
0·0001	125	,,	40	45	0	6	2	4

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and lymph glands were injected into 3 guinea-pigs respectively with negative result.

Control. Two guinea-pigs were inoculated with suspension *B*.

Result. One animal was found dead on 31. v. 25 and at autopsy showed extensive tubercular lesions, *B. tuberculosis* being found. The second animal was found dead on 14. vii. 25 and at autopsy showed severe tubercular lesions, *B. tuberculosis* being recovered.

SUMMARY.

The foregoing report of Professor van Loghem and his co-worker, Miss Benjamins, confirms the results found by Professor Beattie and Dr Lewis, concerning the bactericidal action in milk of the electric current of high voltage. It also proves that the sterilising power of electrical treatment with carbon electrodes is not inferior to that with copper electrodes. These results, and also my experience of the vitamin C sparing effect of the carbon electrodes, have caused me to recommend the use of this kind of electrode in the electric treatment of milk, provided that the milk used be fresh and that it contains but little oxygen. The same reservation applies to milk pasteurised by heat and to pure raw certified milk. It is important that the supply should be fresh.

EXPLANATION OF PLATE VII.

Sections of enlarged micro-photographs of normal and abnormal rib-junctions of guinea-pigs, fed with a standard scurvy-producing diet plus milk in quantities of 30 c.c. per 100 gm. of body-weight, the milk being a raw, pure, Grade A, tuberculin-tested (milk A) or a milk of middling quality (milk B), heat pasteurised or electrically treated with copper electrodes or carbon electrodes.

Fig. 1. The junction is even, the rows of cartilage cells are regularly organised and have normal height. No scurvy.
The animal has been fed on a scurvy-producing diet plus milk A, containing sufficient vitamin C.

Fig. 2. The junction is abnormal, uneven. The rows of cartilage cells are irregular and disorganised. Sub-acute scurvy.
The animal has been fed on the same milk A, electrically treated, with *copper electrodes*.

Fig. 3. Abnormal junction, irregular, uneven. The rows of cartilage cells have disappeared, or are completely disorganised. Between junction and marrow a thick layer of fibrous tissue. Severe scurvy.
The animal has been fed on milk B, not fresh. The vitamin C content was insufficient, therefore 1 per cent. neutralised lemon juice has been added, to make it sufficient. Then treated electrically, with *copper electrodes*.

Fig. 4. Abnormal junction. Strong disorganisation of rows of cartilage cells. Ossified band across the junction, which is irregular and very uneven. Severe scurvy.
The animal has been fed on milk B, mixed with 3 per cent. neutralised lemon juice, and then electrically treated with *copper electrodes*.

Fig. 5. Normal aspect of the junction, which is quite even and regular. The rows of cartilage cells are regular and not shortened. No scurvy.
The animal has been fed on milk B, mixed with 1 per cent. neutralised lemon juice, and then electrically treated with *carbon electrodes*.

Fig. 6. Abnormal junction, very uneven and irregular. Disorganisation of rows of cartilage cells. Acute scurvy.
This animal has been fed on *heat-pasteurised* milk.

(*MS. received for publication* 13. IX. 1926.—Ed.)

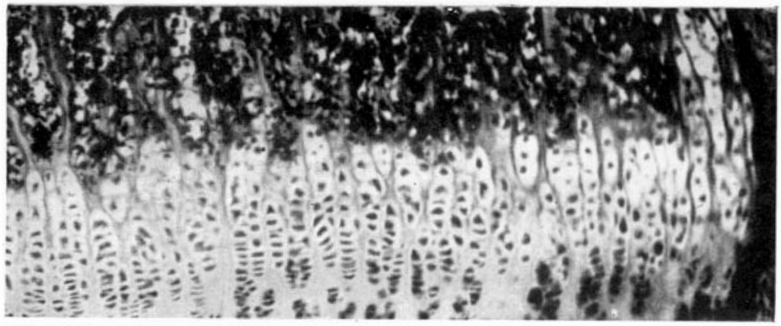

Fig. 1

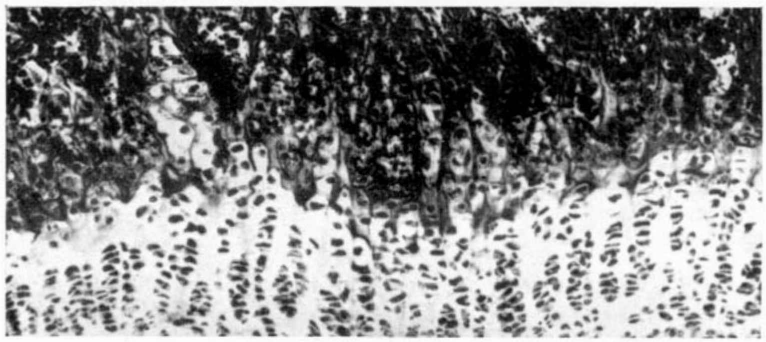

Fig. 2

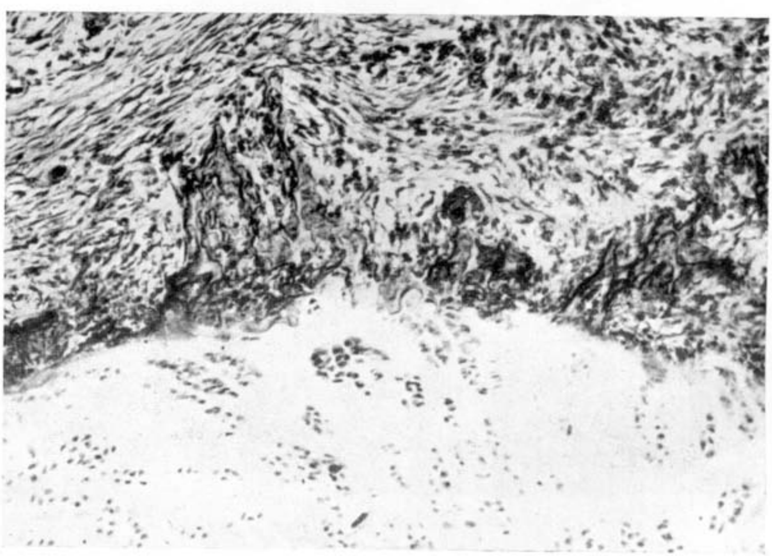

Fig. 3

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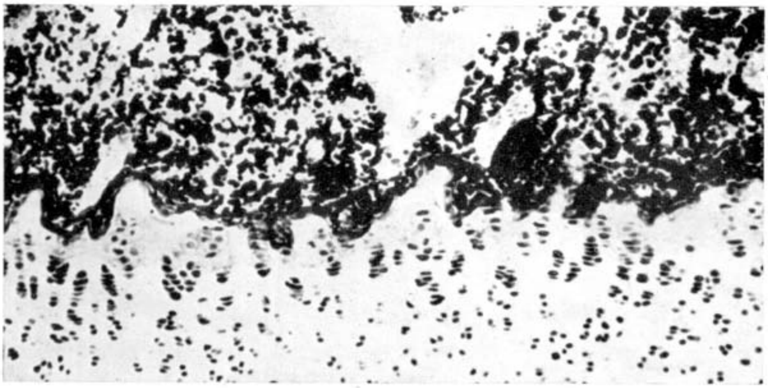

Fig. 4

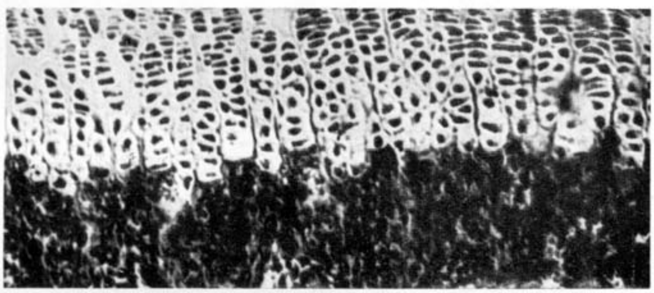

Fig. 5

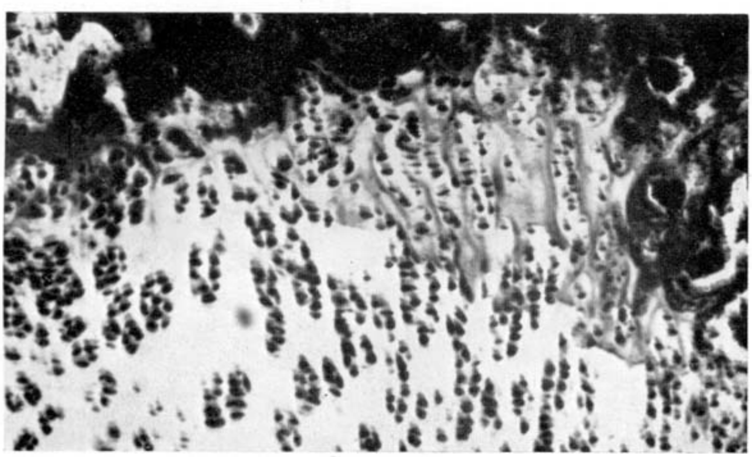

Fig. 6

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