Electrical Current Effects on *E. coli* Growth Rates (36269)

BLAIR A. ROWLEY
(Introduced by J. M. McKenna)
Space Sciences Research Center, University of Missouri, Columbia, Missouri 65201

Studies of the effects of electrical phenomena on growth and metabolism of vegetative cells and biological tissues have shown a definite cause and effect relationship. In 1909 Stone (1) showed that when many genera of bacteria were placed in an electrolytic growth medium with two different metallic plates the current generated through electrochemical reactions stimulated growth of the bacteria. Bacterial counts 100-fold over those of controls were found.

Carlson (2–5) showed that the production of lactic acid, alcohol and gluconic acid was increased 23-33% by several microorganisms in alternating electrical fields of 50 Hz and 7.5-100 mA. Higher levels of current have been reported to exert deleterious effects on bacterial replication and metabolism mainly due to secondary effects. Heat has been implicated by Anderson (6) in the electropure process, and metabolic inhibition of enzyme systems has been shown by Gilliland (7) and Edebo and Selin (8) utilizing high voltage discharges. Edebo and Selin (9) and Edebo *et al.* (10) found that the inhibiting factor was due to photon radiation produced by the electric discharge.

Several cases of inhibition of infection using electric current have been reported. These involved *in vivo* studies of electrical current effects on wound healing. Carey and Lepley (11) showed an inhibition of inflammatory cell infiltration about the negative electrode in the injury site. Wolcott (12) has reported the apparent production of aseptic conditions in infected ischemic skin ulcers after 72 hr exposure to electric current.

There is very little definitive data published on what growth rate changes are produced by electric currents. Depending upon the experimental conditions, it appears that electric current will either enhance or inhibit growth of microorganisms. Because of this apparent enigma, the measurement of growth rate changes produced by electrical currents was undertaken under conditions of minimal electrolysis by-product production.

Materials and Methods. Escherichia coli B was used exclusively in these experiments. Stock seeds of *E. coli* were grown in minimal broth Davis with dextrose and log phase cells were used to initiate the experiments. Platinum–10% iridium was used for the electrodes as no metallic by-products would be produced which might affect cell growth. The electrochemical reaction produces hydrogen gas and hydroxide ions at the cathode and oxygen gas and hydrogen ions at the anode. Hence the cathode area becomes alkaline and the anode area acidic. In the case of direct currents, this condition would continue to build up unless the hydrogen and hydroxide ions migrated together and reacted to form water. The same process would occur using alternating current, however, since each electrode changes from a cathode to an anode periodically, the by-products would combine into water. Hence an alkaline or acidic condition would not persist.

A 3-chambered cylindrical growth cell was built (Fig. 1), which would permit study of growth rate when the bacteria were in the immediate area of the electrochemical reaction and when they were isolated from the electrochemical reaction. In the bottom of the center chamber, 2 air stones were inserted with connecting tees protruding through the base. A standpipe was provided for the center chamber to support a thermometer, to give access to the cells and to prevent froth from overflowing.

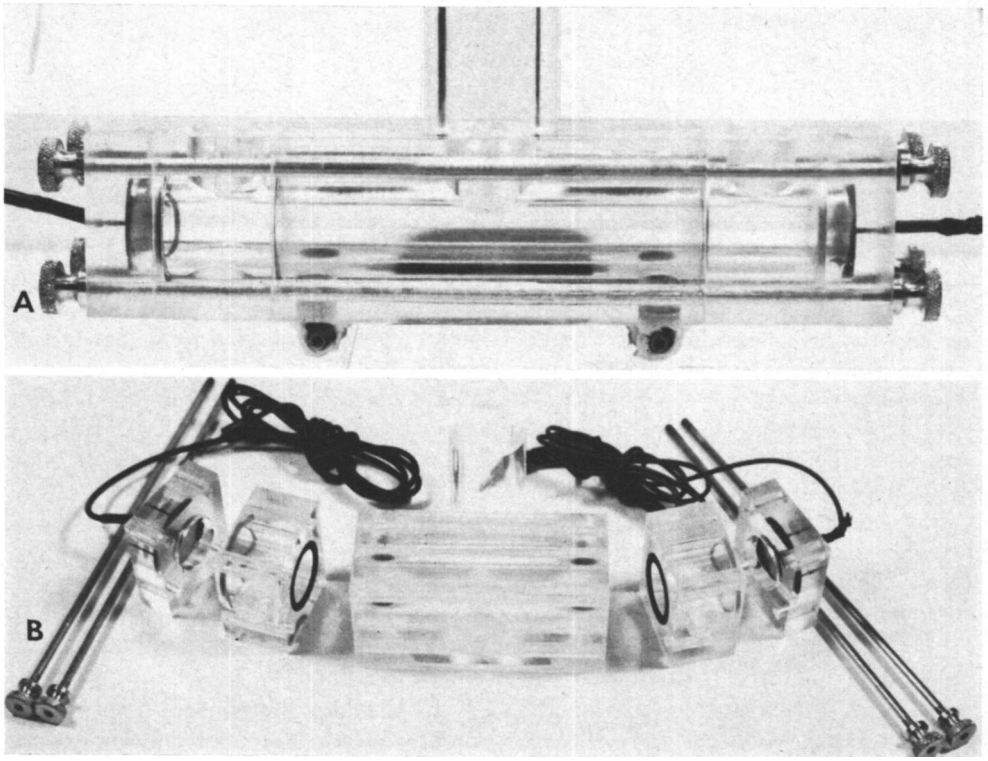

FIG. 1. Growth cell used to apply electrical current to *E. coli*: (A) assembled view; (B) exploded view.

The center chamber inside dimensions were 12.6 cm in length by 3 cm in diameter, having a usable growth volume of 89 ml. Each end chamber had identical dimensions, 3 cm in diameter and 4.5 cm long. The end chambers consisted of 2 sections each. One contained the electrode and the other piece butted up to it like a spacer between the electrode section and the center chamber. The chambers were assembled using flat neoprene gaskets between each section. Between the center chamber and the spacer section a Millipore filter could be inserted across the chamber and held in place by the gaskets. A control chamber was built which was identical in dimensions with the experimental chamber. It served as a standardization growth chamber, run in parallel with the experimental studies.

In order to provide the microorganism with a proper environment, the growth chambers were placed in a constant water bath. The temperature of the water bath was adjusted to maintain the growth media at a temperature of 37°. Cleaned, dried compressed air was supplied by matched calibrated flow reg-

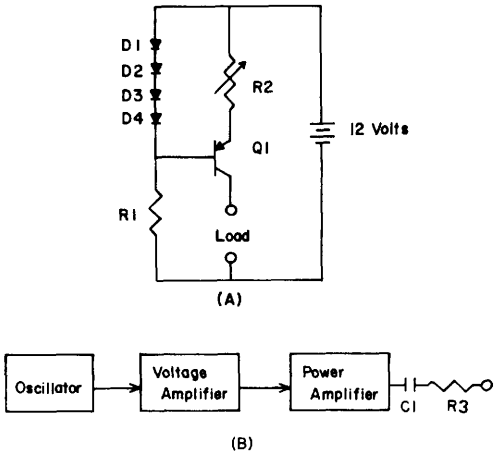

FIG. 2. Circuits used to generate the direct and alternating currents applied to growth cell: (A) microampere direct current generator; (B) block diagram of ac generator.

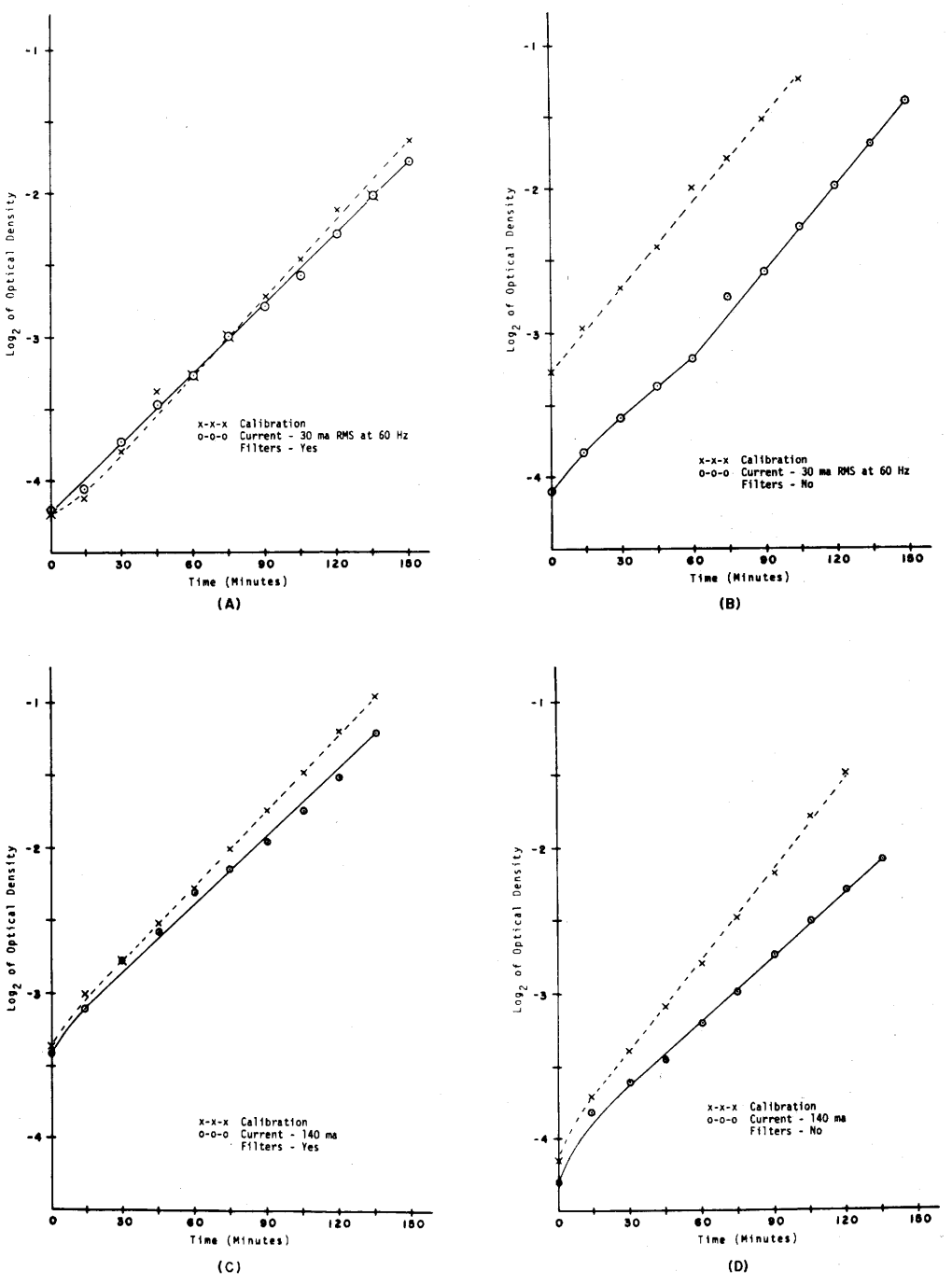

FIG. 3. Growth rate curves of *E. coli* with electrical current applied: (A) 30 mA RMS at 60 Hz with filters; (B) 30 mA RMS at 60 Hz without filter, (C) 140 mA dc with filters; (D) 140 mA dc without filters.

ulators. The flow was set at 1 liter/min, provided stirring with minimal frothing. Generation time was determined turbidimetrically with a Bausch and Lomb Spectronic 20 photometer. Both low level alternating and direct currents were used at 15 and 30 mA at 1, 10, 30, and 60 Hz and 0.2, 1, 14, and 140 mA, respectively.

Generation of the electrical currents required three separate systems. One system produced milliampere direct currents, one produced microampere direct currents, and one produced milliampere alternating currents. The milliampere direct current was obtained using a Hewlett Packard 6200B power supply operating in the constant current mode. The microampere direct current was produced by the circuit shown in Fig. 2A. This circuit produced a very stable low-level current. Its operation was based on the principle of keeping the generator impedance high with respect to the load. In this case the source impedance was the collector impedance of a transistor which can be assumed to be at least 100,000 ohms in the configuration used. The circuitry for generating the alternating current was a mixture of commercial equipment and laboratory built circuitry. Figure 2B is a block diagram of the system.

For the growth rate experiments, medium was poured into the chambers of the two growth cells. To the center chambers was added enough culture seed of *E. coli* already in the logarithmic growth phase to produce a light transmittance in the range of 90%. The percentage transmittance of both chambers was matched within 1 or 2%. The growth chambers were placed in the water bath and the air flow was adjusted to 1 liter/min for both. The electrical current was then applied to the experimental cell and the water bath temperatures were adjusted to produce a temperature of 37° inside the growth chambers. At the start of the run and each 15 min thereafter, 5 ml of growth from each center chamber were pipetted into separate calibrated cuvettes. The transmittance was then recorded, and the samples were returned to their respective chambers.

Results. The curves shown in Fig. 3 are the results obtained at the highest levels with and without filters for both direct current and alternating current. Curves 3A and B, show that alternating current produced little change in growth rate. Direct current produced quite different results. Curve 3C represents results at 140 mA with filters and showed a 14.5% increase in generation time. Curve 3D represents 140 mA with no filters. This yielded a 38.8% increase in generation time.

The results of all experiments are shown in Table I. The influence of alternating current is negligible in most cases. At 60 Hz and 30 mA RMS with filters, the generation time was increased 10.7%. At 30 and 1 Hz both at 30 mA RMS with filters, the generation time was decreased 5.3%. At 10 Hz and 15 mA RMS with filters, the generation time was decreased 3.6%. At 10 Hz and 30 mA RMS without filters, the generation time was increased 5.3%.

The influence of direct current upon generation time was to increase it in most cases. This effect was greater at the higher currents and without filters. The maximum change was an increase of 38.8% at 140 mA without filters. The same current with filters produced a 14.5% increase. At the lower current levels, the effect ranged from a 12.1% increase at 14 mA with filters to no change at 1 mA with filters. One direct current run produced a decrease in the generation time. This was a decrease of 1.8% at 0.2 mA with filters.

During the current applications, the pH was monitored. In the alternating current runs, the pH in all three chambers remained at 7. This was not the case with direct current. As predicted, with filters the anode chamber became acidic and the cathode chamber alkaline while the center chamber remained at a pH of 7. Without filters the pH remained at 7. The alkaline condition in the cathode chamber with filters was the factor which determined the data run length of 150 min. The filter between the cathode chamber and the center chamber became brittle during direct current runs and would rupture after approximately 180 min. This immediately diluted the growth broth and

TABLE I. Generation Times Obtained from Growth Curves of *E. coli*.

Alternating current						Direct current				
Freq. (Hz)	Current (mA RMS)	Filters	Current curve (min)	Cal. curve (min)	Change (%)	Current (mA)	Filters	Current curve (min)	Cal. curve (min)	Change (%)
60	30	Yes	62	56	+10.7	140	Yes	63	55	+14.5
30	30	Yes	53	56	—5.3	14	Yes	65	58	+12.1
10	30	Yes	58	58	0.0	1	Yes	55	55	0.0
1	30	Yes	53	56	—5.3	0.2	Yes	57	56	—1.8
60	15	Yes	55	55	0.0	140	No	68	49	+38.8
30	15	Yes	57	57	0.0	14	No	54	50	+8.0
10	15	Yes	57	55	—3.6	1	No	54	50	+8.0
1	15	Yes	57	57	0.0	0.2	No	53	51	+3.9
60	30	No	51	51	0.0					
10	30	No	56	53	+5.3					
1	30	No	52	52	0.0					

reflected itself as a large shift down in the data curve. To avoid this problem, the curves were not plotted beyond 150 min.

Occasionally, the voltage drop across the cell was also measured as an indication of cell impedance. This varied as would be expected with cell concentration and applied current. The measured impedance varied from 230 to 280 ohms. No phase shift across the chamber was observed on an oscilloscope during the alternating current runs. It was interesting to note that, when the applied direct current was turned off, the cell behaved as a battery with an internal impedance of approximately 1000 ohms. The output voltage would start at 1.25 V and stabilized at 0.75 V with a 20,000 ohm load. This may have been a fuel cell type of reaction as hydrogen and oxygen were separately present over the surface of one or the other platinum electrode due to electrolysis.

Morphologic examination of the bacteria during the runs gave no indication of more than one type of bacteria being present. Gram-stained slides from the liquid culture showed gram-negative rods. Grown on petri dishes, the bacteria produced colonies that were white, circular, and convex with undulate margins.

Summary. The results show that alternating current had little or no effect upon the growth rate of microorganisms in the current and frequency ranges investigated. The general effect of direct current upon generation time was to increase it. When filters were removed, the effect was greater than with filters. This would seem to indicate that the agents causing the decrease in growth rate are a by-product of the electrochemical reaction as they would be more concentrated near the electrodes and without filters the microorganisms would be more exposed.

It appears that this phenomenon of decreased growth rate is not due to a pH change. In all cases, the immediate area in which the cells were grown maintained a buffered condition. This held for both the filtered and nonfiltered runs.

These results agree with the *in vivo* results found in wound healing, that is, the application of electrical current proved deleterious to bacterial growth. This corollary is drawn from a short application time study. There is the possibility that the trend could reverse itself with time and then corollate with Stone's results. However, there is no indication from this study that this would happen. The experimental conditions here were different from Stone's and limited in number of variables.

1. Stone, G. E., Bot. Gaz. (Chicago) **15** (1909).
2. Carlson, S., Arch. Hyg. Bakteriol. **137** (1953).
3. Carlson, S., Arch. Mikrobiol. **20** (1954).
4. Carlson, S., Arch. Hyg. Bacteriol. **144** (1960).

5. Carlson, S., Arch. Microbiol. **37**, (1960).

6. Anderson, A. K., and Finkelstein, R., J. Dairy Sci. **2**, (1919).

7. Gilliland, S. E., and Speck, M. L., Appl. Microbiol. **15**, (1967).

8. Edebo, L., and Selin, I., J. Gen. Microbiol. **50**, (1968).

9. Edebo, L., and Selin, I., J. Gen. Microbiol. **50**, (1968).

10. Edebo, L., Holme, T., and Selin, I., Appl. Microbiol. **17**, (1969).

11. Carey, L. C., and Lepley, D., Surg. Forum **13**, (1962).

12. Wolcott, L. E., S. Med. J. **62**, (1969).

Received Oct. 11, 1971. P.S.E.B.M., 1972, Vol. 139.