OXIDATION-REDUCTION POTENTIALS AND FERRI-CYANIDE REDUCING ACTIVITIES IN PEPTONE CULTURES AND SUSPENSIONS OF ESCHERICHIA COLI

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Oxidation-reduction potentials developed in cultures of *Esch. coli* have been reported by Cannan, Cohen, and Clark (1928), Hewitt (1931) and Boyd and Reed (1931). Boyd and Reed presented evidence that both gaseous (hydrogen) and non-gaseous electromotively active systems are responsible for the potentials observed at different periods of growth in anaerobic cultures of *Esch. coli*. The studies to be reported here were confined to the potentials developed in stationary and continuous flow cultures of *Esch. coli* in peptone, a somewhat less complex medium than those employed by other investigators.

It has been reported (Clifton, 1933a) that broth cultures of *Staphylococcus aureus* in or near the maximum stationary growth phase reduce potassium ferricyanide at a rate in the neighborhood of 6.6×10^{-14} millimols per cell per minute. These studies have been extended to the rates of reduction of ferricyanide at different stages of growth in stationary and continuous flow cultures of *Esch. coli*. Studies on the influence of the concentration of peptone, ferricyanide and organisms on the rate of reduction of ferricyanide in suspensions of "resting" *Esch. coli*, as outlined in a preliminary paper by one of us (Clifton, 1933b), are also presented.

METHODS

Three-necked pyrex distilling flasks of 500 ml. capacity were employed as culture vessels. Two platinum electrodes, sealed

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in soft glass tubing and supported by a rubber stopper, were inserted through the central neck of the flask. A glass tube, extending to the bottom of the flask, was also supported by this stopper. This tube, well plugged with cotton at the upper end, served as an inlet for the compressed air or nitrogen employed in stirring the cultures before samples were removed for pH determinations, for plate counts and for ferricyanide reduction tests. This procedure ensured a thorough mixing of the contents and a greater uniformity in sampling. The side necks of the flasks were plugged with cotton and the system, containing 300 ml. of the culture medium, was sterilized in the autoclave. This half-cell was connected by means of a sterile saturated potassium chloride-agar bridge, supported by a cotton plug in one side neck of the flask, to the standard saturated potassium chloride-calomel electrode.

A similar system (fig. 1) was employed for the continuous flow cultures with the addition of an automatic, constant level siphon supported by the central stopper and a piece of thermometer tubing passing through a stopper in an extension of the side neck opposite the salt bridge. This capillary tube was connected to a peptone supply flask supported above the culture chamber and served to regulate the rate of flow of the solution.

Representative samples of the continuous flow cultures were obtained in the following manner: (1) the outlet tube attached to the small reservoir at the end of the automatic siphon was closed by means of a pinch-cock; (2) the siphon column was broken; (3) the culture was vigorously stirred with air for ten minutes; (4) the siphon was again placed in operation, and (5) a sample was immediately drawn over into the small reservoir at the end of the siphon. The removal of the sample from this receptacle, by means of a pipette, inserted through the sampling tube, reduced the possibilities of contaminating the culture.

The ferricyanide reduction tests were carried out in Pyrex tubes (3 × 20 cm.) to which were attached two side arms. One arm, attached to the bottom of the tube and running to the top where it was bent at a right angle, served as an inlet for the purified nitrogen employed in stirring the sample and maintain-

ing anaerobic conditions. The other arm, attached near the top of the tube, served as an outlet for the gas which escaped under water in a second tube. A platinum electrode and salt bridge were supported by a rubber stopper in the top of the reduction test tube. An opening, normally closed by a glass rod, in this stopper permitted the introduction of the ferricyanide solution after deaeration of the sample.
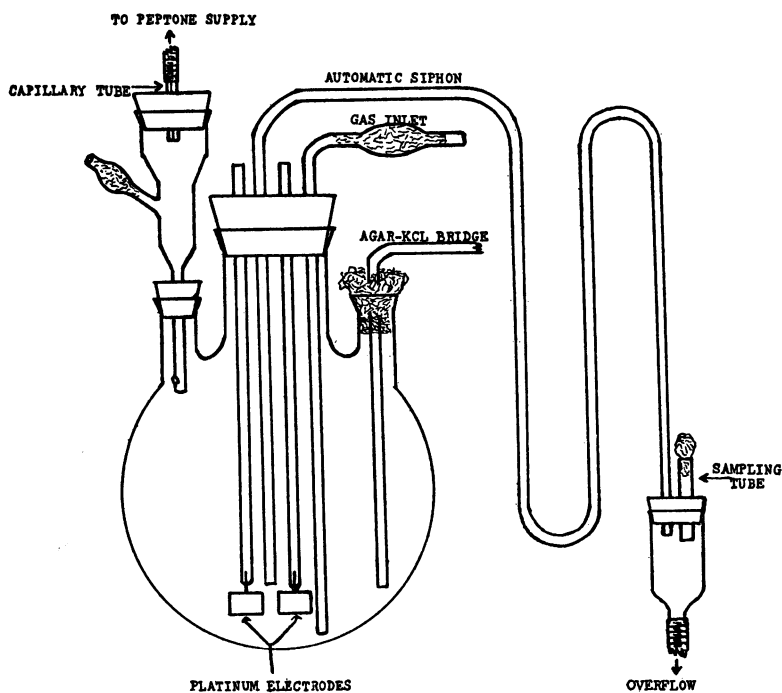

FIG. 1. CULTURE CHAMBER FOR CONTINUOUS FLOW CULTURES

Solutions of Difco peptone in 0.5 per cent saline or in an equivalent concentration of salt plus La Motte's phosphate buffers were employed in these studies. Freshly prepared solutions containing 3 parts of 0.1 molar Baker's C.P. K_3Fe $(CN)_6$ and 1 part of 0.1 molar K_4Fe $(CN)_6 \cdot 3H_2O$ were employed in the ferricyanide reduction tests, the final concentration being 0.01 molar. The E_o of a 0.01 molar ferri-ferrocyanide system in a 1.0 per cent peptone solution at 37.5°C. was +0.391 volt. The concentration of

ferricyanide at any time was determined directly from the potential readings. In the majority of the tests the rate of reduction was calculated from the time required to reduce the ferricyanide from a concentration of 60 per cent to a concentration of 40 per cent. During this time the rate of decrease in ferricyanide concentration with time was quite constant.

The ferri-ferrocyanide system was employed almost exclusively as the oxidation-reduction indicator because (1) it reacts very rapidly with the activated substrate; (2) it does not attack readily other substances in the reaction mixture; (3) it is a readily reversible and highly soluble electromotively active system characterized by a high E_o practically independent of the pH under the conditions of these experiments; (4) the reduced form is not readily oxidized by traces of oxygen, and (5) in general it is better adapted to studies of this nature than the reversible dyestuffs commonly employed in other studies.

Viable counts were made by a standardized dilution and plating technic. All plates were incubated at 37.5°C. for forty-eight hours. The pH determinations were made with a Leeds and Northrop glass electrode assembly at room temperature and were checked at times against quinhydrone electrode and colorimetric determinations. All values reported are rounded off to the nearest 0.1 pH unit.

The time-potential and ferricyanide reduction tests were carried out in an oil bath at 37.5° ± 0.1°C.

EXPERIMENTAL RESULTS

Stationary aerobic cultures

Under aerobic conditions the potential of a 1.0 per cent Difco peptone, 0.5 per cent sodium chloride solution, pH 7.0 was poorly poised in the neighborhood of +0.250 volt. Deaeration of this system with a stream of nitrogen resulted in a negative drift in potential to a value near +0.125 volt.

When 300 ml. of this medium were inoculated with 0.5 ml. of an eighteen-hour peptone culture of *Esch. coli* (laboratory strain K-12) a rapid drop in potential occurred simultaneously with

the period of rapid growth of the organisms and a maximum re-
ducing potential of −0.150 volt was established between the
twenty-fourth and thirty-sixth hour. This maximum reduction
potential was 270 mv. more negative than the potential observed
in sterile deaerated peptone media and about 250 mv. more
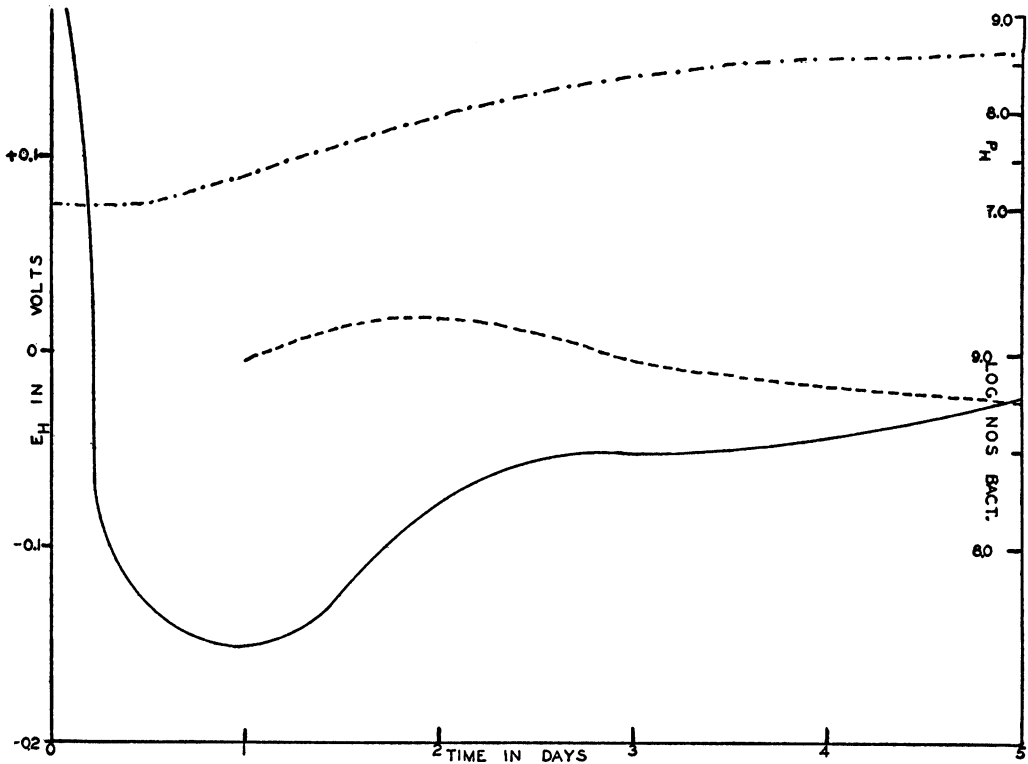negative than the potential observed in a deaerated L 3 Chamber-

FIG. 2. TIME-POTENTIAL (—), TIME-POPULATION (— — —), AND TIME-pH RELA-
TIONSHIPS (.-.-.-) IN AN AEROBIC PEPTONE CULTURE OF *Esch. coli*

land filtrate of a twenty-four hour culture. Typical time-
potential, time-population and time-pH relationships are pre-
sented in figure 2.

In similar tests the cultures were vigorously stirred with *air*
for ten minutes to ensure thorough mixing before samples were
removed for plate counts, pH determinations, and ferricyanide

reduction tests.   During the period of aeration of the cultures a
rapid positive shift in potential of 150 to 200 millivolts was ob-
served but on standing the potential returned, somewhat less
rapidly, to the original level.   Stirring the cultures with nitro-
gen had little effect on the observed potentials but tended to
establish somewhat closer agreement between individual elec-
trodes.

To retard the marked shift in hydrogen ion concentration
observed in these cultures, pH 7.0 to 8.6 by the fifth day, a 1 per
cent peptone solution was weakly buffered at pH 7.0 with phos-
phates to a final concentration of M/50.   The maximum reduc-
ing potential, -0.130 volt, was developed when the culture was
about twenty hours old.   During the next twenty-four hours
the potential shifted to -0.110 volt (pH 7.4) and was maintained
at this level until the culture was discarded on the sixth day
(pH 8.0).

A time-potential relationship similar to that observed with 1
per cent peptone cultures was noted when the peptone concen-
tration was increased to 2.5 per cent.   A maximum reduction
potential of -0.195 volt and a maximum viable population of
110×10^7 per milliliter were developed on the second day.

Nine milliliter samples of the cultures were placed in the re-
duction tubes and deaerated with nitrogen for one-half hour be-
fore the addition of 1 ml. of a freshly prepared 0.075 molar
potassium ferricyanide, 0.025 molar potassium ferrocyanide solu-
tion.   The potential immediately shifted to the E_h level of this
ferri-ferrocyanide system and then decreased with time in a
manner similar to that reported for cultures of *Staphylococcus
aureus*.

The rate of fall in potential ordinarily reached a steady value
within twenty to thirty minutes and was of the order of 0.3 mv.
per minute in a typical twenty-four hour, 1 per cent peptone
culture of *Esch. coli*.   Duplicate tests agreed within 2 per cent
of each other.   The above rate of fall in potential is equivalent
to a total reduction of 0.00028 millimol of ferricyanide per minute.
The total amount of ferricyanide reduced per minute decreased
as the age of the cultures increased and in cultures older than

five to seven days was of such a low magnitude that the rate of change in potential was ordinarily less than 0.04 mv. per minute. Since a sterile solution of peptone reduces ferricyanide at a rate in the neighborhood of 0.02 mv. per minute the results of the ferricyanide reduction tests obtained with the older cultures are only suggestive.

Plate counts carried out before and after the ferricyanide reduction tests indicated that these reagents were not discernibly toxic in the concentration employed during the course of these tests which rarely lasted more than two hours. However, this concentration (total = 0.01 M) of potassium ferricyanide and potassium ferrocyanide does retard growth to some extent in

TABLE 1

Rates of reduction of $K_3Fe(CN)_6$ by 1 per cent peptone cultures of Esch. coli

AGE OF CULTURE	PLATE COUNT PER MILLILITER $\times$ 10^7	MILLIMOLS $K_3Fe(CN)_6$ REDUCED		AVERAGE RATE OF REDUCTION PER CELL PER MINUTE (6 CULTURES)
		Per minute	Per cell per minute	
hours				
24	90	0.00028	3.45×10^{-14}	3.06×10^{-14}
48	146	0.00010	0.76×10^{-14}	1.61×10^{-14}
72	90	0.00009	1.11×10^{-14}	1.18×10^{-14}
96	76	0.00013	1.93×10^{-14}	1.95×10^{-14}
120	60	0.00005	0.92×10^{-14}	1.00×10^{-14}

young cultures. The results of the reduction tests with samples from a typical culture are recorded in table 1, together with the averages of the results obtained with 6 similar cultures.

In the majority of the tests the highest rate of reduction per cell per minute was observed in twenty-four-hour cultures. This rate of reduction tends to decrease as the age of the culture increases, although individual variations were observed, particularly around the fourth day.

During the period of rapid growth the rate of reduction of ferricyanide (0.001 M mixture), as calculated from Buchanan's (1930) formula, ranged from 2.45×10^{-13} to 5.62×10^{-14} millimols per cell per minute.

A behavior similar to that observed with 1.0 per cent peptone cultures was noted when the peptone concentration was increased

to 2.5 per cent. However, the rate of reduction in the 2.5 per cent peptone culture was greater than that observed in the 1 per cent peptone cultures. Typical results are given in table 2.

In another experiment a 1.0 per cent peptone solution in equal amounts of $M/15$ phosphates (pH 7.4) and 0.5 per cent saline solutions was employed as the culture medium. After twenty-four hours growth at 37.5°C. the culture reduced ferricyanide at a rate of 4.44 $\times$ 10^{-14} millimols per cell per minute. When a 1:1 dilution of the ferri-ferrocyanide mixture was employed the rate of reduction decreased to 3.84 $\times$ 10^{-14} millimols per cell per minute. When the concentration of cells was reduced to one-half the original value by dilution with saline the rate of reduction

TABLE 2

Rates of reduction of $K_3Fe(CN)_6$ in 2.5 per cent peptone cultures of Esch. coli

AGE OF CULTURE	PLATE COUNT PER MILLILITER $\times$ 10^7	MILLIMOLS $K_3Fe(CN)_6$ REDUCED	
		Per minute	Per cell per minute
hours			
24	80	0.00053	7.36 $\times$ 10^{-14}
48	110	0.00051	5.15 $\times$ 10^{-14}
72	83	0.00036	4.82 $\times$ 10^{-14}
96	73	0.00029	4.41 $\times$ 10^{-14}
144	51	0.00018	3.92 $\times$ 10^{-14}

was 4.03 $\times$ 10^{-14} millimols per cell per minute. The addition of 1.0 ml. of a 10 per cent peptone solution to a sample similar to the above increased the rate of reduction to 9.09 $\times$ 10^{-14} millimols per cell per minute. Similar results were obtained with older cultures and are summarized in table 3.

These and similar results show that small dilutions of the cultures ordinarily increased to a slight extent the rate of reduction of ferricyanide. The same behavior was noted in suspensions of "resting" bacteria. The addition of fresh peptone to the samples markedly increased the rate of reduction. In the above test when 0.1 ml. of 10 per cent peptone was added to 9.0 ml. of the culture the increase in peptone concentration was only from 1.0 to 1.1 per cent but the rate of reduction increased nearly nine-

fold. This suggests that there may be a small fraction of the peptone that is most readily assimilated by the cells.

A sample of this buffered twenty-four-hour culture was tested for its reducing activity after twenty-four and forty-eight hours incubation at 3 to 5°C. The rate of reduction of the original twenty-four-hour culture was 4.44×10^{-14} and after twenty-four and forty-eight hours in the refrigerator was 4.61 and 4.06×10^{-14} millimols per cell per minute, respectively. The plate counts were 55, 53, and 52×10^7 per ml.

TABLE 3

The effect of dilution and of peptone concentration on the rate of reduction of $K_3Fe(CN)_6$ by a buffered (M/30 phosphates, pH 7.4) culture of Esch. coli

AGE OF CULTURE	pH OF CULTURE	PLATE COUNT PER MILLILITER	10 PER CENT PEPTONE ADDED	MILLIMOLS $K_3Fe(CN)_6$ REDUCED PER CELL PER MINUTE IN	
				9 ml. culture	4.5 ml. culture
hours			*cc.*		
24	7.5	55×10^7		4.44×10^{-14}	4.03×10^{-4}
			1.0		9.09×10^{-4}
48	7.65	128×10^7		0.96×10^{-14}	1.56×10^{-4}
			0.5		11.98×10^{-4}
			1.0		17.36×10^{-4}
72	7.8	130×10^7		0.68×10^{-14}	1.02×10^{-4}
			0.1	5.64×10^{-14}	
			1.0		16.24×10^{-4}

CONTINUOUS FLOW CULTURES

The automatic siphons in the continuous flow culture flasks were adjusted to retain 250 ml. of the 1.0 per cent peptone solution in the flasks and capillary tubes were selected to give a daily flow of approximately 500 and 250 ml. in experiments CF 1 and CF 2, respectively.

During the first twenty-four hours of growth in the continuous flow cultures, seeded with 0.5 ml. of an eighteen-hour peptone culture of *Esch. coli*, the potential decreased approximately 400 mv. A slow negative potential drift continued for eight to ten days and reached a level 50 to 75 mv. more negative than the maximum reduction potentials developed in the stationary (typical aerobic) cultures. The E_h was maintained at this low

level until the cultures were discarded on the twenty-fourth day.

A maximum viable population was established within five to eight days. The value for the viable count dropped slowly after the tenth day and concomitantly the rates of flow of the peptone solutions decreased due to the accumulation of suspended matter
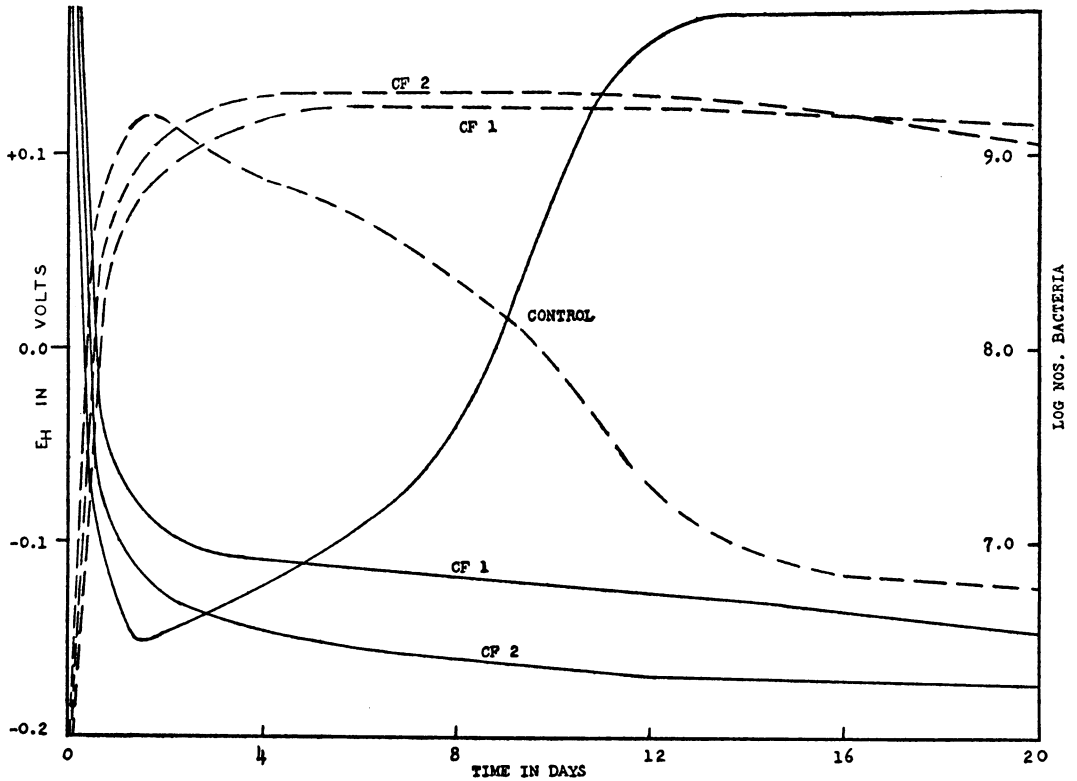

FIG. 3. TIME-POTENTIAL (—) AND TIME-POPULATION RELATIONSHIPS (— —) IN CONTINUOUS FLOW PEPTONE CULTURES OF *Esch. coli*
CF 1, 500 ml.; CF 2, 250 ml. daily flow and in a stationary culture (control).

in the capillaries. The peptone supply flasks were disconnected on the twenty-fourth day at which time the rates of flow had decreased to less than one-half of the original values.

The potentials and counts then shifted in a manner similar to those observed in the stationary (ordinary aerobic) cultures

after maximum populations had been established. In these continuous flow cultures the pH remained quite constant in the neighborhood of 7.6. A similar behavior was observed in eight separate tests and in one test with 2.5 per cent peptone. The time-potential and time-population relationships for the continuous flow cultures 1 and 2 are graphically recorded in figure 3.

TABLE 4

Rate of reduction of $K_3Fe(CN)_6$ in constant flow cultures of Esch. coli

CULTURE	AGE	PLATE COUNT PER MILLILITER $\times 10^7$	MILLIMOLS $K_3Fe(CN)_6$ REDUCED	
			Per minute	Per cell per minute
	hours			
	24	66	0.00016	2.62×10^{-14}
	48	87	0.00045	5.48×10^{-14}
	72	94	0.00042	4.93×10^{-14}
1.0 per cent peptone (500 ml. daily)	120	170	0.00059	3.84×10^{-14}
	168	168	0.00054	3.57×10^{-14}
	240	162	0.00044	3.05×10^{-14}
	460	129	0.00044	3.74×10^{-14}
	576	142	0.00071	5.58×10^{-14}
	24	79	0.00024	3.40×10^{-14}
	48	115	0.00041	3.94×10^{-14}
	72	130	0.00034	2.89×10^{-14}
1.0 per cent peptone (250 ml. daily)	120	190	0.00042	2.43×10^{-14}
	168	190	0.00040	2.34×10^{-14}
	240	164	0.00037	2.51×10^{-14}
	460	102	0.00050	5.44×10^{-14}
	576	88	0.00016	2.03×10^{-14}
	24	50	0.00040	8.88×10^{-14}
	48	132	0.00060	5.05×10^{-14}
2.5 per cent peptone (250 ml. daily)	96	134	0.00057	4.71×10^{-14}
	120	138	0.00096	7.73×10^{-14}
	144	150	0.00099	7.33×10^{-14}

Ferricyanide reduction tests with the continuous flow cultures were carried out in the manner previously described on 9 ml. samples drawn from the body of the well stirred cultures. Typical results are given in table 4.

These results show a general tendency on the part of the continuous flow cultures to maintain a higher rate of reduction per

cell per minute than was observed with the stationary cultures. Also a higher rate of reduction was observed when either the peptone concentration or the rate of flow of the peptone was increased.

In a continuous flow 1.0 per cent peptone culture, 0.0075 and 0.0025 molar with respect to potassium ferricyanide and potassium ferrocyanide, respectively, the E_h slowly fell from an initial value of $+0.421$ volt to $+0.312$ volt on the fourth day at which time the viable population was only 19×10^7 per milliliter. This concentration of potassium ferri- and ferrocyanide was apparently slightly toxic to the actively growing cells. In a constant flow culture containing only one-tenth of the above concentration of ferri- and ferrocyanides the potential slowly fell through the range of this system during the early stages of growth and reached an E_h level of $+0.035$ volt and a population of 176×10^7 viable cells per milliliter on the third day. This was followed by a slow positive drift to an E_h of $+0.075$ volt and a population of 170×10^7 on the fifth day at which time the culture was discarded. From these results it is apparent that *Esch. coli* can build up a high viable count at an E_h level 200 to 300 mv. more positive than that developed in the ordinary cultures.

SUSPENSIONS OF "RESTING" ESCH. COLI

Forty-eight hour cultures of *Esch. coli*, grown on nutrient agar at 37.5°C., were suspended in saline and washed 3 times by centrifugation. The organisms were then suspended in a mixture of equal parts of M/15 phosphate buffer, pH 7.5, and physiological saline to give a standard turbidity corresponding to a total count of approximately 10×10^9 cells per ml. These suspensions were incubated for two hours at 37.5°C. in shallow sterile dishes in order that the cells might utilize any residual foodstuffs.

Measured volumes of suspensions of these "resting" bacteria, were introduced into the reduction tubes and appropriate amounts of buffer, saline and peptone solutions were added. These suspensions were deaerated for one-half hour before the addition of the standard ferri-ferrocyanide solution. In all tests

the concentration of phosphate buffer and of saline was maintained constant. The concentration of reagents employed in a typical test are as follows: 1.0 ml. of a 20 per cent peptone solution in physiological saline, 2.0 ml. of physiological saline solution, 5.0 ml. of M/15 phosphate buffer solution, pH 7.5, 10.0 ml. of the bacterial suspension in the buffer-saline mixture and

TABLE 5

Influence of the concentration of peptone, oxidant and organisms on the rate of reduction of $K_3Fe(CN)_6$ by a suspension of "resting" Esch. coli, pH 7.5

TOTAL VIABLE COUNT $\times 10^9$	CONCENTRATION OF PEPTONE	INITIAL CONCENTRATION OF $K_3Fe(CN)_6$	MILLIMOLS $\times 10^{-14}$ $K_3Fe(CN)_6$ REDUCED PER CELL PER MINUTE
Influence of peptone concentration			
	per cent	*millimols*	
18.0	10	0.150	3.09
18.0	5.0	0.150	3.95
18.0	3.0	0.150	4.30
18.0	2.0	0.150	4.61
18.0	1.0	0.150	4.33
18.0	0.5	0.150	3.66
18.0	0.1	0.150	1.33
Influence of initial ferricyanide concentration			
18.0	1.0	0.225	6.17
18.0	1.0	0.150	4.33
18.0	1.0	0.075	2.72
18.0	1.0	0.037	1.28
Influence of bacterial concentration			
27.0	1.0	0.150	3.70
18.0	1.0	0.150	4.33
9.0	1.0	0.150	5.50
1.8	1.0	0.150	8.83

2.0 ml. of a 0.1 molar solution of potassium ferricyanide (3 parts)—potassium ferricyanide (1 part). The results of a typical experiment illustrating the influence of different concentrations of peptone, ferricyanide and viable cells on the rate of reduction of ferricyanide are recorded in table 5.

The results obtained with suspensions of "resting" *Esch. coli*

show that the rate of reduction of ferricyanide increased with increase in the concentration of peptone (other variables being maintained constant) up to 2.0 or 3.0 per cent and then decreased as the concentration of peptone was further increased.   The rate of reduction of ferricyanide by well-washed "resting" *Esch. coli* in the absence of peptone or by peptone in the absence of "resting" bacteria was negligible.   Duplicate tests agreed within 1.0 per cent of each other and similar tests with suspensions prepared at different times showed the same general behavior and gave results of the same order of magnitude.

The rate of reduction of ferricyanide in a 1.0 per cent peptone solution decreased as the concentration of bacteria was increased. Under the same conditions the rate of reduction of the ferricyanide increased as the original concentration of the ferricyanide was increased.   A decrease in temperature of 15°C. reduced the rate of reduction of ferricyanide to approximately one-half of the value observed at 37.5°C.

The hydrogen ion concentration of the suspension exerted a slight influence on the rate of reduction of ferricyanide.   Between the pH limits studied, 7.0 and 8.0, the maximum rate was observed at pH 7.5.   In a typical experiment the number of millimols $\times$ 10^{-14} of ferricyanide reduced per cell per minute was 5.58, 5.75, and 5.50 at pH 7.0, 7.5, and 8.0, respectively.

In order to study the effect of dead cells on the rate of reduction of ferricyanide a suspension containing 36 $\times$ 10^8 viable cells per ml. was irradiated with ultra-violet ight.   These light-killed cells were added to portions of the original suspension and the rate of reduction of ferricyanide determined under conditions as outlined in table 6.

These and similar results indicate that the presence of dead cells has little or no influence on the rate of reduction of ferricyanide.

Various peptone preparations were employed in other tests on the rate of reduction of ferricyanide in identical suspensions of *Esch. coli*.   The observed rates of reduction of ferricyanide in millimols $\times$ 10^{-14} per cell per minute at pH 7.5 are as follows:

Bacto-tryptone (Difco) 1.0 per cent solution...................... 5.8
Protease-peptone (Difco) 1.0 per cent solution.................... 5.8
Peptone (Difco or Merck) 1.0 per cent solution................... 4.8
Neopeptone (Difco) 1.0 per cent solution......................... 4.1
Bacto-protone (Difco) 1.0 per cent solution...................... 2.6

DISCUSSION

The oxidation-reduction potential developed in a 1.0 per cent peptone solution under aerobic conditions is poorly poised in the neighborhood of +0.250 volt (pH 7.0) and on deaeration with a stream of nitrogen gas slowly falls to an equilibrium value near +0.125 volt. During the period of active growth of *Esch. coli* under ordinary aerobic conditions a characteristic drop in po-

TABLE 6

Influence of light-killed cells on the rate of reduction of $K_3Fe(CN)_6$ in a 1.0 per cent peptone solution, pH 7.5

VIABLE CELLS ADDED	KILLED CELLS ADDED	RATE OF REDUCTION OF $K_3Fe(CN)_6$ MILLIMOLS $\times 10^{-14}$ PER VIABLE CELL PER MINUTE
cc.	*cells*	
10		3.88
10	5	3.88
5		4.00
5	5	3.94

tential of 400 to 500 mv. is observed. The maximum reducing potential developed in or near the maximum stationary growth phase of the culture is considerably more negative than that developed in either a deaerated peptone solution or in the filtrate (L 3 Chamberland) of a portion of the culture. Vigorous aeration of the culture results in the temporary establishment of a more positive potential while stirring with nitrogen has little effect on the observed E_h. A positive drift in potential is observed, concurrent with a decrease in the viable count as the age of the culture increases.

In the stationary cultures the rate of reduction of ferricyanide per cell per minute tends to decrease with increasing age of the cells. The results obtained with suspensions of "resting" coli

(tables 5 and 6) suggest that the rate of reduction should increase as the number of viable cells decreases. Some evidence of this is observed in the data presented for the older cultures. The general tendency for the rate of reduction of ferricyanide per cell per minute to decrease with increasing age of the culture may be due to the depletion of readily utilizable foodstuffs (evidence of which has been presented in this communication) to the effect on the cells of the accumulation of waste products, and to physiological differences between cells of different ages.

During the most active period of growth of *Esch. coli* the rate of reduction of ferricyanide ranged from 2.45×10^{-13} to 5.62×10^{-14} millimols per cell per minute. Martin (1932) reported an oxygen consumption of the same order of magnitude (4.0×10^{-13} to 4.0×10^{-14} millimols per cell per minute) during the early stages of growth of *Esch. coli* and likewise noted a tendency for the oxygen consumption per cell to decrease with increasing age of the culture. Walker and Winslow (1932) reported a carbon dioxide production of 0.5×10^{-14} millimol per cell per minute during the period of stable population in aerated peptone cultures of *Esch. coli*, a value of the same order of magnitude as that observed for the reduction of ferricyanide in similar cultures.

In the continuous flow cultures a maximum reduction potential, somewhat greater than that observed in the stationary cultures, is developed and maintained. The viable count also remains at a maximum stationary level, somewhat higher than the maximum developed in the stationary cultures. The rates of reduction of ferricyanide in the continuous flow cultures are, in general, quite constant and greater than those observed in the stationary cultures. In these continuous flow cultures fresh peptone solution is supplied constantly, and rapidly mixes with the culture while an equivalent amount of the culture is continuously removed. We may conclude that the cells in the continuous flow cultures are on the average metabolically similar to those in the latter phases of active growth in ordinary cultures.

The mechanism for the development of the potentials observed in peptone cultures of *Esch. coli* may be postulated as follows: As growth begins an aerobic type of metabolism occurs

for a varying time during which the bacteria utilize oxygen and other oxidizing agents in the media. As the concentration of these reagents is decreased and the total metabolic activities of the cells increase, the potential falls and an anaerobic type of metabolism predominates in the culture. The potential reaches a maximum reducing value when the total metabolic requirements of the cells are at a peak value and all available oxidation-reduction systems are employed to meet these demands. In continuous flow cultures this potential is maintained for the above reasons, in stationary cultures a positive shift occurs as the metabolic requirements decrease. The mechanism for the development of reducing conditions as outlined above is in agreement with that postulated by Boyd and Reed (1931) and Hewitt (1933).

The data presented in this communication suggest a possible explanation for the decrease with time in the rate of growth of bacteria and the inability of the bacterial population to increase above a rather definite maximum under a given set of conditions. The total amount of ferricyanide reduced per unit time increases as the concentration of bacteria is increased, other variables being maintained constant. However, this increase is not proportional to the increase in bacterial population, and the amount of ferricyanide reduced *per cell per minute* actually decreases as the concentration of bacteria is increased.

This behavior is also illustrated by the decrease, with increasing age of the culture, in the rate of reduction of ferricyanide per cell during the early phases of growth and to some extent after a maximum population has been established. Approximately 3×10^6 molecules of ferricyanide are reduced per cell per minute in a culture in or near the maximum stationary growth phase while as many as 15×10^6 or more molecules may be reduced per cell per minute in or near the logarithmic growth period. A similar tendency is noted in the reports in the literature on the rate of oxygen consumption, carbon dioxide production, etc., in cultures of *Esch. coli* and other organisms.

The rate of reduction of ferricyanide per cell increases as the concentration of ferricyanide or of peptone is increased. This

again is not proportional to the concentration of ferricyanide or of peptone and holds only within certain limits because high concentrations of ferricyanide may exert a toxic action and concentrations of peptone above 3.0 per cent reduce the rate of reduction.

These considerations suggest that the rate of the metabolic activities per cell under a given set of conditions is controlled by the concentration of foodstuffs, oxidants, and cells. The maximum activity per cell is noted when sufficient foodstuffs and oxidant are sorbed and activated (if necessary) in unit time to provide, upon interreaction, for the maximum respiratory and metabolic requirements of the cells.

The change, increasing with age of a culture, in the rate of metabolic activity of the cell may be interpreted on a probability basis. As the number of bacteria increases the concentration gradient of foodstuffs and of oxidants between the cell and its environment decreases, and therefore, the probability of sufficient materials being available per cell per unit time decreases. This decrease may account for the decrease in rate of growth observed in cultures of bacteria in which a relatively high population has been established. An increase in concentration of foodstuffs or oxidant increases, within limits, the probability of sufficient energy and building material being available per cell per unit time.

Other factors (Rahn, 1932) play important rôles in regulating the time-growth relationships in bacterial cultures. The hypothesis presented in this communication does, however, present a relatively neglected aspect of certain of the factors involved in controlling the growth of bacteria.

SUMMARY

Growth, oxidation-reducton potentials, and ferricyanide reduction have been studied in stationary and continuous flow peptone cultures of *Esch. coli*.

A marked fall in potential occurs during the period of rapid growth and a maximum reduction potential is developed in or near the maximum stationary growth period. This potential

and maximum viable count is maintained quite constant in continuous flow cultures.

The concentrations of peptone, organisms and ferricyanide play closely connected rôles in controlling the metabolic activities of the cells, as measured by the rate of reduction of ferricyanide.

Evidence is presented which suggests that the oxidation-reduction potentials observed in bacterial cultures are a resultant of the metabolic activities of the cells.

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