RELATIONSHIP BETWEEN "CHLORAMPHENICOL REDUCTASE ACTIVITY" AND CHLORAMPHENICOL RESISTANCE IN ESCHERICHIA COLI[1, 2]

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Smith and Worrel (1950) have reported that the major pathway employed by *Escherichia coli* for the inactivation of the bacteriostatic effects of chloramphenicol was the reduction of the nitro group on the chloramphenicol molecule to an amino group. A fairly extensive study of the enzymatic reduction of chloramphenicol has been made by Smith and Worrel (1949). This study, however, involved only chloramphenicol sensitive strains of bacteria, and no attempt was made at that time to determine what effect the enzymatic reduction had on the chloramphenicol resistance mechanism.

We have reported previously (Merkel *et al.*, 1951) on the *in vitro* development of chloramphenicol resistance in strain B of *E. coli*. By the method of repeated contacts with graded amounts of the antibiotic it was possible to develop cultures which could grow in brain-heart infusion broth containing 2 mg of chloramphenicol per ml of medium.

The present paper demonstrates a correlation between the enzymatic reduction of chloramphenicol ("chloramphenicol reductase activity") by *E. coli*, strain B, and the development of chloramphenicol resistance. The relative instability of the "highly" resistant cultures has made it possible to study the relationship between resistance and "chloramphenicol reductase activity" during the loss of resistance as well as during the development of resistance, and has shown that this correlation exists only at certain stages in the resistance.

MATERIALS AND METHODS

Strains. The chloramphenicol resistant strains of *E. coli* used in the present study are those which were developed by Merkel *et al.* (1951) in a previous investigation. *E. coli*, strain 100, refers to a culture "trained" to grow in 100 μg per ml of chloramphenicol ("chloromycetin")[4] in brain-heart infusion medium, and, similarly, *E. coli*, strain 2000, refers to the highly resistant culture "trained" to grow in 2 mg per ml of chloramphenicol in the same medium. *E. coli*, strain B, refers to the parent sensitive strain.

Media. All cultures were "trained" in brain-heart infusion medium (Difco), and all determinations of "chloramphenicol reductase activity" were made in either this medium, Monod's salt-glucose medium (Monod and Wollman, 1947), or in a specially prepared synthetic medium referred to in table 1 as the "chloramphenicol reductase" medium.

Measurements. All measurements of growth were made with a Klett-Summerson photoelectric colorimeter using the red no. 66 filter.

pH measurements were made with a Beckman, Model G, pH meter.

Polarographic results were obtained with a manually operated Fisher Elecdropode[5] which was equipped with a mercury stand-tube, saturated calomel reference electrode, KCl-agar

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bridge, an electrolytic cell prepared by cutting the length of a 13 by 100 mm test tube to approximately 50 mm, and a constant temperature water bath. Solutions of methyl red and basic fuchsin were used as maxima suppressors, and oxygen-free nitrogen was used to remove oxygen from the test solutions.

Determination of "chloramphenicol reductase activity." The particular culture for which the "chloramphenicol reductase activity" was to be determined was inoculated (0.1 ml of an 18 to 24 hr culture) into 20 ml of brain-heart infusion medium and allowed to incubate for 15 to 18 hours in the absence of chloramphenicol. Each culture was harvested then by centrifugation and washed two times with cold Sorensen's M/15 phosphate buffer at pH 7.35 or pH 7.42. After the last washing the cells were resuspended in 6 ml of the so-called "chloramphenicol reductase" medium, or in brain-heart infusion medium, or in Monod's synthetic medium containing 100 μg of chloramphenicol per ml of medium. Where a constant number of cells was desired, a turbidity of 50 (approximately 1 mg of cells per 5 ml of medium) was chosen arbitrarily. The cultures were kept in an ice bath until all dilutions were complete. Then these cultures were placed in a constant temperature water bath at 37 C along with control tubes containing only the media plus chloramphenicol. These tubes were incubated for various periods of time (chloramphenicol-cell contact time). At the end of the designated incubation periods the turbidities of the suspensions were again checked; the solutions were chilled in an ice bath, and the majority of the cells were removed by centrifugation at 5,000 rpm for 10 minutes. The amounts of unreduced chloramphenicol remaining in the supernatants were determined either by the turbidimetric bioassay method of Joslyn and Galbraith (1950) or by the polarographic method described below. If the chloramphenicol was determined by the turbidimetric bioassay method, the supernatants first were sterilized by filtration through sintered glass. This precaution was not necessary if the polarographic method of analysis was used.

TABLE 1

Composition of "chloramphenicol reductase" medium (nitrogen-free)

	mg/100 ml
Na_2HPO_4	767.0
KH_2PO_4	435.6
$MgSO_4 \cdot 7H_2O$	19.1
$CaCl_2$	1.0
$FeSO_4 \cdot 7H_2O$	0.054
Glucose	36.0
Chloramphenicol	10.0

pH adjusted to 7.42.

The large number of assays which were required made it almost impossible to utilize the turbidimetric bioassay procedure for routine analyses. The polarographic technique described below was used for the majority of the chloramphenicol determinations and was checked periodically by the bioassay method. Generally, good agreement was obtained between the two methods of assay. It appeared, however, that the polarographic procedure was more sensitive for the determination of minimum and maximum ranges of chloramphenicol reduction. Consequently, the reduction of chloramphenicol by growing cultures of *E. coli*, strain 100, was detected at the end of a shorter incubation period than was detected by the bioassay procedure, and by the polarographic procedure the reduction was shown to proceed at a somewhat gradual rate rather than the logarithmic rate observed with the bioassay method. The early measurement of reduced chloramphenicol by the polarographic procedure was checked also by a colorimetric procedure and was found to be real. The colorimetric method involved the addition of an HCl-ethanol solution of *p*-dimethylaminobenzaldehyde to the culture filtrates. Very small amounts of reduced chloramphenicol immediately produced a yellow color in the solutions.

Polarographic method of assay. The polarographic technique used in the present experiments does not differ essentially from the technique developed by Hess (1950) and from standard procedures described by Kolthoff and Lingane (1947). Since the polarographic method proved to be invaluable for the many assays of chloramphenicol which had to be performed in the present experiments, it might be of benefit to describe in brief the particular procedure used in these studies.

After the majority of the bacterial cells were removed from the chloramphenicol culture media by centrifugation, the pH of 3 ml portions was adjusted to 7.2 with minute amounts of 1:1 HCl or 30 per cent NaOH. The solutions

were poured into the specially prepared electrolytic cell and placed in a constant temperature water bath at 25 ± 0.5 C. A drop of methyl red (1:3,000 in alcohol) was added to the brain-heart solutions, and a drop of basic fuchsin (0.1 per cent in 10 per cent alcohol) was added to the synthetic solutions as maxima suppressors. Oxygen-free nitrogen was bubbled through the electrolytic cell for five minutes to remove dissolved oxygen from the solutions. Polarograms (current-voltage curves) were obtained then by varying the applied E.M.F. from 0.1 to 0.9 of a volt and recording the uncalibrated galvanometer deflections. A capillary electrode having a drop time of 3 seconds was used in all the determinations. The heights of the polarographic waves (i_d), which are proportional to the concentrations of the reducible substances, were measured by the extrapolation method (Kolthoff and Lingane, 1947). The chloramphenicol concentrations were determined from standard i_d-concentration curves which had been obtained under exactly the same experimental conditions as above.

RESULTS

Preliminary experiments in which the amount of reduced chloramphenicol was determined by the turbidimetric bioassay procedure indicated that if growth in the culture media described in the previous section was started with small inocula of *E. coli*, chloramphenicol reduction did not occur until late in the log phase of growth. When brain-heart infusion medium containing 100 μg per ml of chloramphenicol was inoculated with 0.1 ml of an 18 hour culture of *E. coli*, strain 100, diluted to a turbidity of 25, maximum reduction was not obtained until the culture had been incubated for 8 to 10 hours. This could lead to the assumption that the reduction of chloramphenicol by *E. coli* was merely a secondary process of resistance. There was, however, another possibility; namely, that small-scale, cellular reduction was occurring continuously in growing cultures but the reduction could not be detected until either a large amount of cells was present in the media or the cells were left in contact with the chloramphenicol for long periods of time. The likelihood of this possibility was increased by results which indicated that the reduction was an intracellular process or, at least, a reaction which required the presence of bacterial cells; culture filtrates would not reduce chloramphenicol.

With the possibility of small-scale reductions in mind three types of experiments utilizing whole cell suspensions were planned, which would magnify essentially the reactions occurring with small inocula. These involved studies on the effects of cell numbers, culture age, and chloramphenicol-cell contact time on the "chloramphenicol reductase activity".

These experiments were performed in both nutrient medium (brain-heart infusion broth) and in the nitrogen-free "chloramphenicol reductase" medium. The use of the nutrient medium was discontinued after several experiments because of the variability in the growth rates of *E. coli* cultures having different degrees of resistance.

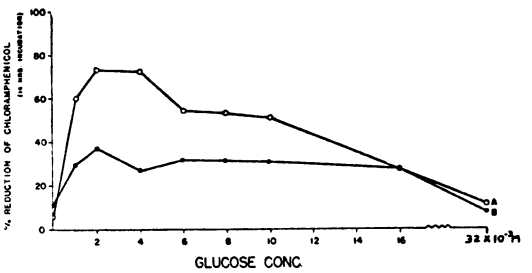

Figure 1. Effect of glucose concentration on the "chloramphenicol reductase activity" of 6½ hour cultures of the partly resistant *Escherichia coli*, strain 100 (A), and on the parent sensitive *E. coli*, strain B (B). "Chloramphenicol reductase activity" measured polarographically in nitrogen-free synthetic medium containing 100 μg per ml of chloramphenicol. Chloramphenicol-cell contact time of 14 hours.

The decision to adopt the nitrogen-free "chloramphenicol reductase" medium was arrived at after it was discovered that maximum reductase activity in Monod's synthetic medium lacking a nitrogen source was dependent on the glucose concentration in the medium (figure 1). Maximum reduction with *E. coli*, strain 100, was obtained between 2 to 4 × 10^{-3} M glucose, whereas *E. coli*, strain B, gave maximum reduction over the range 2 to 16 × 10^{-3} M glucose.

Maximum reductase activity was exhibited between pH 7 to 8 for both the resistant and sensitive cultures.

The results recorded below have been obtained with the "chloramphenicol reductase" medium described earlier, but several results obtained with brain-heart infusion medium are

included for comparison. All determinations of chloramphenicol in these results were obtained by the polarographic method. The data in figures 1 to 3 represent averages of at least three experiments.

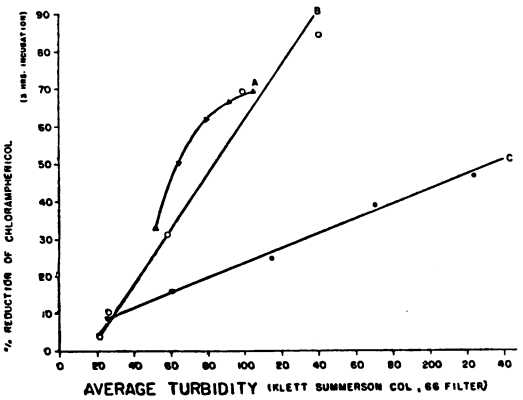

Figure 2. Effect of cell numbers on the "chloramphenicol reductase activity". (A) 18 hour culture of *Escherichia coli*, strain 100, in brain-heart infusion medium; (B) 16½ hour culture of *E. coli*, strain 2000, in the nitrogen-free "chloramphenicol reductase" medium; (C) 16½ hour culture of *E. coli*, strain B, in the "chloramphenicol reductase" medium. Chloramphenicol-cell contact time of 3 hours.

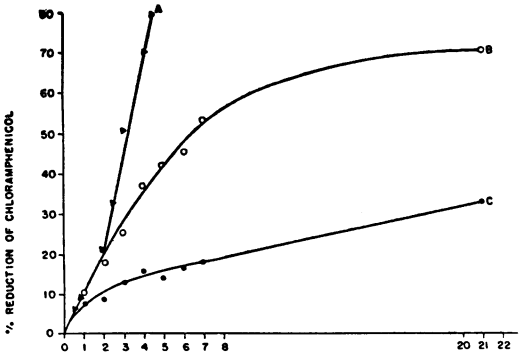

Figure 3. Effect of chloramphenicol-cell contact time on "chloramphenicol reductase activity". (A) 6 to 10 hour cultures of *Escherichia coli*, strain 100, in brain-heart infusion medium; (B) 15 hour culture of *E. coli*, strain 2000, in the nitrogen-free "chloramphenicol reductase" medium; (C) *E. coli*, strain B, similar treatment as (B).

Using both the "chloramphenicol reductase" medium and brain-heart infusion medium we have found that, within the limits of 2 to 12 hours, the culture age did not affect appreciably the "chloramphenicol reductase activity". However, cultures above 24 hours old when tested for reductase activity in brain-heart infusion medium and above 48 hours when tested in the "chloramphenicol reductase" medium had lost a considerable amount of both reductase activity and chloramphenicol resistance.

Figures 2 and 3 show clearly that "chloramphenicol reductase activity" varies directly with the concentration of cells and with the chloramphenicol-cell contact time.

In each of these experiments it can be noted that the resistant cultures always exhibit greater reductase activity than the parent sensitive cultures. It also appears from these results that chloramphenicol reduction is a continuous process in growing cultures but is not measurable until either a large number of cells is present in the media or until the cells have been left in contact with the chloramphenicol for long periods of time. Maximum reductase activity must result from a combination of these two factors, and the maximum activity cannot be attributed solely to culture age.

An attempt was made then to see if there was a correlation between the development of increased resistance in *E. coli* and an increase of "chloramphenicol reductase activity." The results in table 2 indicate that the first step in chloramphenicol resistance is associated with a comparable rise in the "chloramphenicol reductase activity".

Resistance was developed in these particular studies by inoculating a series of tubes containing 5 ml of brain-heart infusion medium and increasing amounts of chloramphenicol with the parent sensitive *E. coli*, strain B, and incubating for 18 hours at 37 C. At the end of 18 hours the cells were harvested by centrifugation from the highest concentration of the antibiotic permitting fair growth (turb. >25) and diluted with Sorensen's M/15 phosphate buffer (pH 7.35) to a turbidity of 25. Then 20 ml of fresh brain-heart infusion medium with no added chloramphenicol were inoculated with 0.1 ml of the cell suspension. At the same time a new series of tubes, containing 5 ml of brain-heart infusion medium with increasing concentrations of antibiotic, was inoculated with 0.5 ml of the cell suspension to repeat the above process at the next higher level of resistance. The cells in the 20 ml volumes of medium were harvested at the end of 15

hours and tested for chloramphenicol sensitivity and "chloramphenicol reductase activity" by the procedures described under materials and methods. The only variation in the procedure used for measurement of "chloramphenicol reductase activity" was the use of different chloramphenicol-cell contact times as indicated in table 2. Chloramphenicol sensitivity tests were performed with known concentrations of chloramphenicol on all of the above 15 hour cultures by the turbidimetric bioassay procedure referred to earlier. Sensitivities have been recorded as 50 per cent inhibition concentrations (amount of chloramphenicol in brain-heart infusion medium needed to inhibit the growth of a culture to 50 per cent of the growth of that same culture in the absence of antibiotic).

Maximum reductase activity, under the conditions of the experiments, was reached at relatively low resistances (note italicized values of table 2). The maxima reached depended on the rate of reduction of chloramphenicol and on the chloramphenicol-cell contact time. The rate of reduction is a measure of the quantity of enzyme present in the cells and is apparently proportional to resistance. In figure 3 it was shown that chloramphenicol reduction varied with the chloramphenicol-cell contact time, and the maximum reduction with a nongrowing culture was obtained in 8 hours. Table 2 contains 3 experiments in which 3, 6, and 14 hours' chloramphenicol-cell contact times were used. Since the amount of reduction was dependent on the contact time, the reductase results with 14 hours' contact appear to be discontinuous while 6 and 3 hours' contact show a continuous rise in both resistance and in reductase activity. Apparently with the longer contact time the effect of culture resistance is overshadowed.

An attempt to raise the maximum amount of reduction by using anaerobic conditions was unsuccessful. Complete aerobic conditions, however, greatly inhibited the reductase activity. Another inhibitory factor was the tendency for the cells of resistant cultures to clump together. This latter effect tended to give lower values for the reductase activity of *E. coli*, strain 2000, cultures as compared to cultures with lower resistances (table 3).

Although polarographic determinations on the amount of chloramphenicol reduced by a single culture were reproducible to ±2 per cent, slight variations in the results reported in tables 2 and 3 are expected since each result was obtained with an individual culture carried through the procedure described earlier for the determination of resistance and reductase activity.

The chloramphenicol resistance developed in cultures of *E. coli* was found to be relatively unstable. On subculturing in brain-heart in-

TABLE 2

Relation between gain of chloramphenicol resistance and "chloramphenicol reductase activity"*

CHLORAMPHENICOL-CELL CONTACT TIME AT 37 C							
14 hr		6 hr		3 hr		6 hr	
50% inhibition†	% red.‡	50% inhibition	% red.	50% inhibition	% red.	50% inhibition (back transfer of (a))	% red.
(*μg/ml*)							
0.13	26.0	0.16	17.8	0.16	9.1	0.57	66.0
0.15	22.3	0.18	28.4	0.19	10.5	0.55	74.5
0.26	*64.1*	0.28	43.0	0.28	19.2	*0.41*	*66.4*
0.46	57.5	*0.48*	*59.7*	0.46	43.4	0.31	48.8
0.66	62.1	0.57 (a)	60.2	*0.64*	*55.2*	0.27	26.7
1.04	55.6			1.25	55.7	0.20	13.6
1.20	58.3			2.20	58.0	0.14	18.7

* *Escherichia coli*, strain B, "trained" in brain-heart infusion medium.

† Amount of chloramphenicol necessary to inhibit the growth of a culture to 50 per cent of that of the same culture grown in the absence of the antibiotic (chloramphenicol resistance).

‡ Per cent reduction of chloramphenicol by approximately 1 mg of cells (turbidity 50) in nitrogen-free "chloramphenicol reductase" medium.

fusion medium in the absence of antibiotic, resistance was rapidly lost. By this method of subculturing in the absence of antibiotic, cultures of varying degrees of resistance and "chloramphenicol reductase activity" were obtained and the correlation between resistance and reductase activity could be studied at various levels of resistance.

When a slightly resistant culture (note back-transfer (a) in table 2) was subcultured in the absence of antibiotic, resistance and reductase activity were lost by almost the same pattern as in the development of this "first step" in resistance.

at this particular level has proved to be stable even after 28 subcultures in antibiotic-free media.

A more thorough discussion concerning the nature of the loss of chloramphenicol resistance is presented in a subsequent publication (Herrmann and Steers, 1953).

DISCUSSION

"Chloramphenicol reductase activity" could either be a primary resistance mechanism or it could merely be one of the secondary results of developed resistance. If *E. coli* were able to grow in the presence of chloramphenicol simply because it had the ability to inactivate the anti-

TABLE 3

Correlation of loss of chloramphenicol resistance with loss of "chloramphenicol reductase activity"*

NO. OF BACK-TRANSFERS OF	I (14 HR)		II (14 HR)		III (6 HR	
	50% inhibition	% red.†	50% inhibition	% red.	50% inhibition	% red.
Escherichia coli, strain 2000						
0	1,100	66.1	920	46.3	950	53.1
1	—	—	800	57.7	—	—
2	870	64.8	710	64.8	—	—
3	890	68.5	430	55.6	—	—
4	550	64.8	225	52.9	—	—
5	19.3	45.5	25	27.4	200	42.4
6	22.5	43.0	10.8	17.6	—	—
7	9.1	26.7	8.4	17.6	21.2	37.9
10	—	—	7.4	21.7	13.7	12.7
15	—	—	—	—	12.5	15.5
Escherichia coli, strain B	—	—	0.1	33.1	0.1	19.5

* Loss of resistance by the highly resistant strain, *Escherichia coli*, strain 2000, when subcultured in brain-heart infusion medium without chloramphenicol.

† Per cent reduction of chloramphenicol by approximately 1 mg of cells (turbidity = 50) in the "chloramphenicol reductase" (nitrogen-free) medium.

On subculturing *E. coli*, strain 2000, in brain-heart infusion medium without chloramphenicol, both resistance and reductase activity dropped off rapidly, but a stage was reached where the resistance remained above that of the parent sensitive cultures, yet the "chloramphenicol reductase activity" actually dropped below that of the parent sensitive cultures (table 3). Table 3 lists a representative number of numerous similar experiments conducted over the period of one year. In each experiment the break in the correlation between the loss of resistance and the loss of reductase activity occurred at approximately the same value of resistance (50 per cent inhibition concentration of 10 to 20 μg of chloramphenicol per ml of medium). The resistance

biotic by reduction, growth in a concentration of chloramphenicol which was normally inhibitory should be associated with a marked reduction of chloramphenicol. Also, if the reductase activity is associated with resistance, variances in resistance should accompany variances in reductase activity.

The possibility that the reduction of chloramphenicol was taking place continuously in inoculated media in a cellwise manner, and was not being detected until the cell numbers increased to a point where a measurable amount of chloramphenicol was being reduced, made the first contention difficult to realize experimentally. Since no techniques were available to study the single-cell reactions, experiments were designed

to magnify these reactions. This, of course, was done with the reservation that the results obtained with large numbers of organisms may not only have enlarged the cellular reactions but may also have added new effects as a result of intercellular reactions. One of the most pronounced effects of large numbers of organisms, aside from the metabolite or coenzyme contributions, is to cause the lowering of oxygen tension in the medium. This possible effect has been minimized considerably since anaerobic conditions applied to either lightly or heavily inoculated media did not produce greater amounts of reduction than that obtained under ordinary conditions.

The data presented indicate that the reduction of chloramphenicol plays a primary role in the resistance mechanism of this strain of *E. coli*. This contention is supported further by the data demonstrating a direct relationship between the gain and loss of resistance and the increase and decrease of "chloramphenicol reductase activity" at various levels of resistance. One particular level or stage of resistance which has been described in the results does not uphold this relationship.

Two factors which have a direct bearing on the nature of the resistance should be pointed out. The first of these is the fact that maximum reductase activity is reached at relatively early stages in the development of resistance, yet the resistances of the cultures can be developed to much higher stages; secondly, on subculturing the partly resistant *E. coli* (table 2) in the absence of antibiotic both resistance and reductase activity fall off in about the same pattern as that by which they were developed. However, when the highly resistant *E. coli*, strain 2000, is subcultured in the absence of antibiotic, the loss of resistance and reductase activity parallel each other for a certain number of back-transfers, but a point is reached where the resistance remains above that of the parent sensitive cultures, yet the reductase activity falls below the normal sensitive level. Although the correlation between reductase activity and resistance was not studied over the entire range of resistances during the development of the *E. coli*, strain 2000, a review of the pattern of the development of its resistance in brain-heart infusion medium indicated a definite break or "step" at the same point (10 to 20 μg per ml 50 per cent inhibition) where the break in the correlation between resistance and reductase activity occurred during the back-transfer procedure.

In view of the above results it appears as if chloramphenicol resistance is developed in this strain of *E. coli* by two or more mechanisms either simultaneously or at different stages of resistance. There exists a correlation between resistance and reductase activity during the development and the loss of resistance at low levels. Starting at high levels of resistance the loss of resistance and the loss of reductase activity follow the same general pattern on subculture in the absence of the antibiotic; however, a particular stage of resistance at a 50 per cent inhibition of 10 to 20 μg of chloramphenicol per ml of medium blocks the complete reversion to the parent sensitive culture. The reductase activity at this level of resistance is almost 50 per cent less than that of parent sensitive cultures. This particular stage in resistance seems to indicate a genetic step. At low levels of resistance ("first step") the pattern of adaptation seems to best fit the picture. In other words, we seem to have adaptation to a certain level in resistance followed by a genetic change. This may actually be occurring simultaneously and may occur at various levels of resistance.

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SUMMARY

Data are presented which illustrate the possible role of "chloramphenicol reductase activity" in the primary mechanism by which *Escherichia coli* becomes resistant to chloramphenicol.

High concentrations of glucose in "nitrogen-free" media have been shown to be inhibitory to the "chloramphenicol reductase activity" of resistant cultures.

A direct correlation has been shown between chloramphenicol resistance and "chloramphenicol reductase activity" in *E. coli* at certain stages of resistance; however, this activity does not seem to account for all of the resistance which can be developed.

REFERENCES

HERRMANN, E. C., JR., AND STEERS, E. 1953 Studies on the mechanism of the loss of chloramphenicol resistance in *Escherichia coli*. J. Bact., **66**, 397–403.

HESS, G. B. 1950 Polarographic estimation of chloramphenicol (Chloromycetin). Anal. Chem., **22**, 649–651.

JOSLYN, D. A., AND GALBRAITH, M. 1950 A turbidimetric method for the assay of antibiotics. J. Bact., **59**, 711–716.

KOLTHOFF, I. M., AND LINGANE, J. J. 1947 *Polarography*. 2nd edition. Interscience Publishers, New York.

MERKEL, J. R., HERRMANN, E. C., AND STEERS, E. 1951 A study of chloramphenicol resistance in *Escherichia coli*. Bact. Proc., **1951**, 55–56.

MONOD, J., AND WOLLMAN, E. 1947 L'inhibition de la croissance et de l'adaptation enzymatique chez les bacteries infectees per le bacteriophage. Ann. inst. Pasteur, **73**, 937–956.

SMITH, G. N., AND WORREL, C. S. 1949 Enzymatic reduction of chloramphenicol (Chloromycetin). Arch. Biochem., **24**, 216–223.

SMITH, G. N., AND WORREL, C. S. 1950 The decomposition of Chloromycetin (chloramphenicol) by microorganisms. Arch. Biochem., **28**, 232–241.