PROBABLE ABSENCE OF DIRECT INDUCTION OF BACTERIAL RESISTANCE TO STREPTOMYCIN

TSUTOMU WATANABE, TOSHIO FUKASAWA, AND DAIZO USHIBA

Department of Bacteriology, Keio University School of Medicine, Tokyo, Japan

Received for publication December 17, 1956

The origin of acquired microbial resistance to various drugs has been discussed by many workers in recent years. Although most of the geneticists and bacteriologists interested in this subject have been inclined to support the theory of spontaneous mutation and selection, a few workers have insisted that the direct induction of bacterial resistance by drugs themselves should be taken into consideration. Several excellent means, such as the fluctuation test of Luria and Delbrück (1943) and Demerec (1948), the spreading experiment of Newcombe (1948), and the replica plating technique of Lederberg and Lederberg (1952), seem to give proof that spontaneous mutation and selection are the cause of drug resistance. Nevertheless, it has been difficult to rule out entirely the possible role of an inducing effect of drugs (Hinshelwood, 1949; Sevag, 1946; and Abraham, 1953). In recent years, several experimental reports have been presented which claim to demonstrate the inheritance of induced adaptive processes in bacteria, and mutagenic effects of drugs on the development of resistance in bacterial populations (Linz, 1950; Eagle *et al.*, 1952; Akiba, 1952, 1953a, 1953b; Yokota, 1955a, 1955b, 1955c, 1955d, 1955e; Kanai *et al.*, 1953).

Akiba and his co-worker Yokota have carried out extensive studies on the origin of drug resistance, especially with streptomycin, and have presented an "induction theory." According to this theory, the mutagenic action of the drugs plays a major role in the emergence of bacterial resistance to most drugs; spontaneous mutation and selection are regarded as subsidiary factors. Akiba first reported that the mutagenic action of streptomycin is specific (1953a) but later changed his theory, reporting that it is as nonspecific as other mutagens (Yokota, 1955d). Their experimental results may be summarized as follows: (1) During the growth of a bacterial population in the presence of even nonselective concentrations of streptomycin, the bacteria acquire a

high resistance to this antibiotic. (2) In the presence of threshold concentrations of streptomycin, high resistance is developed (also reported by Eagle *et al.*, 1952). (3) In the "resting state," the organisms develop streptomycin resistance when they are kept in contact with the drug in the absence of bacterial multiplication (i. e., eliminating selection by the drug).

Akiba's experiments caused much interest among bacterial geneticists, including the present authors who tried to confirm his results. In previous papers (Ushiba and Watanabe, 1954; Watanabe, 1954a, 1954b), the present authors reported that Akiba's experimental results, (1) and (2), are essentially similar to each other and should be explained on the basis of "differential selection due to differential inhibition" which is based on the theory of spontaneous mutation and selection. In other words, the more resistant cells are less affected than the less resistant ones in the presence of certain concentrations of streptomycin, thus causing a selective growth of more resistant cells from which more and more resistant mutants arise. Attempts to reproduce Akiba's "resting cell" experiment in numerous trials with *Salmonella enteritidis* strain 11 were unsuccessful. It was suggested that nutrients released by streptomycin killed sensitive cells may have supported the multiplication of resistant mutants in Akiba's experiments with supposedly resting cells.

Szybalski (1954) has also carried out similar experiments with *Escherichia coli* strains K-12 and B/r and *Salmonella typhimurium* and confirmed that the majority of washed and starved cells become streptomycin resistant when incubated for about two weeks at 37 C in phosphate buffer containing this antibiotic. Since this streptomycin resistance was found by recombination and transduction analysis to be of nuclear origin, he tentatively concluded that it may be considered to be the first fully documented case of "Lamarckian" inheritance in bacteria, by

which he meant nuclear inheritance of a single, purposefully acquired character. He furthermore assumed that this may be due to more or less specific chromosomal damage associated with the loss of a dominant factor controlling streptomycin sensitivity. His paper received considerable attention in recent reviews of Zelle (1955) and Eagle and Saz (1955).

The present authors initially failed in obtaining any increase of streptomycin resistance in "resting cell" experiments with *S. enteritidis* strain 11, but subsequent studies with *E. coli* strain K-12 yielded positive results. It was so surprising to find that the cells of the latter strain could acquire streptomycin resistance in the apparent absence of cell division and selection that it was decided to undertake more critical and analytical studies.

Newcombe and McGregor (1951) had previously tested for possible mutagenic effects of streptomycin by determining the mutation rates to "full streptomycin resistance" from intermediate resistance, both in the presence and absence of the drug, and reported that the antibiotic has a slight, but nonspecific mutagenic action on bacteria. In this regard, it was suggested in previous papers (Watanabe, 1954a, 1954b) that Newcombe and McGregor's (1951) class of "full resistance" should actually be classified into at least two genotypically distinct classes. When a strain of intermediate resistance is grown in the presence of even a small amount of drug, highly resistant cells could be developed following stepwise increase of resistance, and, although these highly resistant mutants phenotypically resemble single step highly resistant mutants, both mutants could be differentiated genotypically. The present authors have called the single step highly and fully resistant cells and the multistep resistant cells "streptomycin indifferent" and "streptomycin resistant" respectively. The former type may correspond to Mitchison's type 3a (1953). On the basis of this classification, the mutagenicity experiments of Newcombe also were reinvestigated in the present studies.

MATERIALS AND METHODS

E. coli strain K-12 was employed in the "resting cell" experiments. Sixteen-hr-old cultures of this organism grown in nutrient broth, on nutrient agar or in minimal broth were centri-

fuged and washed three to five times with phosphate buffer (K_2HPO_4 , 7 g; KH_2PO_4 , 2 g; distilled water, 1 L). The washed cells were resuspended in buffer at various concentrations. Some suspensions were immediately used and others kept at 37 C overnight for the purpose of starving the cells. The washed cell suspensions were not aerated, because it was found that this treatment killed a considerable portion of the "resting cells." The viable cells in these suspensions were assayed by diluting and culturing on nutrient agar with or without streptomycin (1,000 μ g/ml). Aliquots of 4.5 ml were then distributed in separate tubes. Dihydro-streptomycin sulfate (Takeda) was added to each of these samples in varying concentrations, except for the antibiotic free control suspension. All samples were kept for more than two weeks at 37 C; in a few experiments they were kept in a refrigerator. After this period of time the cells were thoroughly washed and again assayed on nutrient agar with and without streptomycin.

In the mutagenicity experiments, *S. enteritidis* strain 11 was used. This strain was used in preference to *E. coli* strain K-12 because immune serum for K-12 was not available at the time of the experiments to differentiate mutants from possible contaminants by slide agglutination test. Fluctuation tests were conducted according to Luria and Delbrück (1943) both in the absence and presence of 0.5 μ g/ml of streptomycin, and the assay for streptomycin resistance at the end of incubation was carried out on nutrient agar containing 5,000 μ g/ml of the antibiotic. Further, in order to differentiate between streptomycin indifference and dependence, each of the colonies was subcultured on nutrient agar with or without streptomycin (1,000 μ g/ml). For the calculation of the mutation rate, only "method 1" of Newcombe was applicable, since the growth rate of streptomycin indifferent cells of this strain was found to be significantly lower than that of sensitive cells.

RESULTS

"Resting cell" experiments with *E. coli* strain K-12. As in Szybalski's report (1954), in some experiments a majority of the cells survived both in the presence and absence of streptomycin in the buffer, whereas all of the cells were completely killed in the presence of the drug in other experiments even with thoroughly washed and

TABLE 1

Effects of streptomycin on the development of streptomycin resistance in washed and starved cells of Escherichia coli strain K-12

No. of Viable Cells in Inoculum	No. of Streptomycin Indifferent Cells in Inoculum*	Streptomycin Concentration in Buffer	No. of Cells after Incubation at 37 C for 18 Days	
			On nutrient agar with no streptomycin	On nutrient agar with 1,000 μ g/ml streptomycin
		μ g/ml		
2.3×10^9 /ml	1.4/ml	0	2.5×10^8 /ml	0/ml
2.3×10^9	1.4	5	1.6×10^7	8×10^2
2.3×10^9	1.4	10	2.3×10^7	1.1×10^7
2.3×10^9	1.4	25	2.8×10^7	2.4×10^7
2.3×10^9	1.4	50	0	0
2.3×10^9	1.4	100	8.8×10^7	9.4×10^7
2.3×10^9	1.4	500	0	0
2.3×10^8	0†	50	0	0
2.3×10^7	0	50	0	0
2.3×10^6	0	50	0	0

* One ml each of serial dilutions of the initial inoculum was poured into a petri dish with nutrient agar containing 1,000 μ g/ml of streptomycin. Number of indifferent colonies was calculated from the results on duplicate plates.

† Theoretically, 0.14 cells per ml.

starved cells. Some of the results obtained are shown in table 1. In all of the experiments, when no streptomycin indifferent or dependent mutants were present in the initial inocula, all of the "resting cells" were killed. Although the mutation rate of K-12 to streptomycin indifference is reported to be around 10^{-10} , mutants can be found occasionally in much higher frequencies in certain populations depending upon the time when the mutation took place. In the presence of streptomycin concentrations below 5 μ g/ml, only some of the cells were "converted" to streptomycin indifference. On the other hand, no change in streptomycin sensitivity was observed in populations maintained as control "resting cells" in streptomycin free suspensions.

In some of the experiments, all of the streptomycin resistant cells that arose from "resting cells" were found to be streptomycin dependent. This seems rather difficult to understand on the basis of the direct induction theory. Such results could have been due, however, to the propagation of spontaneously occurring streptomycin dependent mutants already present in the initial inocula.

It was concluded, therefore, that streptomycin exhibits a bactericidal effect even on thoroughly washed, apparently resting cells and that the

presence of some spontaneously arising streptomycin indifferent cells in the initial inocula is essential for the development of streptomycin indifferent cells in antibiotic containing buffer. It appears possible that the sensitive cells are killed by the drug and that the initially present streptomycin indifferent cells multiply by utilizing the nutrients released from the killed cells. The following experiments were conducted to investigate this possibility.

"Resting cell" experiments with a mixture of sensitive cells and T 1 resistant, streptomycin indifferent cells of *E. coli* strain K-12. The mutant used was selected by exposing a large number of sensitive cells to T 1 and subsequently to a high concentration of streptomycin. Both sensitive and mutant cells were mixed in proportions shown in table 2, and the "resting cell" experiment was carried out as previously described. The results (table 2) indicated that in these experiments also the majority of the cells kept in contact with sufficient concentrations of streptomycin were "converted" to indifference. It was found that practically all of the indifferent cells obtained were T 1 resistant, while in the absence of the drug most of the cells harvested at the end of the experiments were shown to be T 1 and streptomycin sensitive. These results seemed to

support further the above mentioned assumption of the role of selection among so-called resting cells.

In *E. coli* strain K-12, however, the possibility of genetic recombination cannot be overlooked, even though it has been reported that the transfer of F-agent (Lederberg *et al.*, 1952) does not occur in the resting state (Hayes, 1953). Therefore, additional reconstruction experiments were performed with F (-) strains (Hayes, 1953).

"Resting cell" experiments with mixtures of two F (-) auxotroph strains. The auxotrophic strains were obtained by Dr. Hsu in the authors' de-

partment with the penicillin screening method as modified by Adelberg and Myers (1953). One of them, mutant K 7-1, requires both arginine and histidine, while the other mutant K 6-70, is dependent on both tryptophan and proline. Both strains were found to be F (-). In the first experiment shown in table 3, cells of strain K 6-70 marked with T 1 resistance and streptomycin indifference were mixed with T 1 and streptomycin sensitive cells of strain K 7-1 in the proportions shown in the table. "Resting cell" experiments with this mixture showed that in the presence of 50 $\mu\text{g/ml}$ of streptomycin the

TABLE 2

Effects of streptomycin on the development of streptomycin resistance in a mixture of sensitive and T 1 resistant, streptomycin indifferent cells of Escherichia coli strain K-12 (washed and starved cells)

Experiment No.	No. of Sensitive Cells in Inoculum	No. of T 1 Resistant, Streptomycin Indifferent Cells in Inoculum	Streptomycin Concentration in Buffer	No. of Cells after Incubation at 37 C for 18 Days	
				On nutrient agar with no streptomycin	On nutrient agar with 1,000 $\mu\text{g/ml}$ of streptomycin
1	$4.1 \times 10^8/\text{ml}$	$6.1 \times 10^2/\text{ml}$	$\mu\text{g/ml}$		
	4.1×10^8	6.1×10^2	50 0	$3 \times 10^6/\text{ml}$ 6.8×10^7	$4 \times 10^6/\text{ml}$ 0
2	3.7×10^9	1.3×10^3	50	1.5×10^6	1.6×10^6
	3.7×10^9	1.3×10^3	0	2×10^8	20

TABLE 3

Effects of streptomycin on the development of streptomycin resistance in a mixture of two differently marked auxotrophs of Escherichia coli strain K-12 (washed and starved cells)

Experiment No.	Strain and No. of T 1 and Streptomycin Sensitive Auxotroph Cells	Strain and No. of T 1 Resistant, Streptomycin Indifferent Auxotroph Cells	Streptomycin Concentration in Buffer	No. of Cells after Incubation at 37C for 16 Days	
				On nutrient agar with no streptomycin	On nutrient agar with 1,000 $\mu\text{g/ml}$ of streptomycin
1	K 7-1 (A - H -)	K 6-70 (T - P -)	$\mu\text{g/ml}$		
	$7 \times 10^9/\text{ml}$	$1.1 \times 10^2/\text{ml}$	50	$7.1 \times 10^7/\text{ml}$	$7.8 \times 10^7/\text{ml}$
	K 7-1 (A - H -)	K 6-70 (T - P -)	0	6×10^7	1.6×10^2
	$7 \times 10^9/\text{ml}$	$1.1 \times 10^2/\text{ml}$	50	0	0
2	K 7-1 (A - H -)	K 6-70 (T - P -)			
	$7 \times 10^9/\text{ml}$	0	50	4.5×10^6	3.2×10^6
	K 6-70 (T - P -)	K 7-1 (A - H -)	50	9×10^9	2.7×10^2
	9×10^9	K 7-1 (A - H -)	0	1.3×10^8	0
	K 6-70 (T - P -)	K 7-1 (A - H -)			
	9×10^9	2.7×10^2	50	0	0
	K 6-70 (T - P -)	K 7-1 (A - H -)	50	9×10^9	2.7×10^2
	9×10^9	0	0	0	0

A -, arginineless; H -, histidineless; T -, tryptophanless; P -, prolineless.

Figure 1. Assays of stored, washed and starved cells of *Escherichia coli* strain K-12. I, colony counts on streptomycin free nutrient agar of cells incubated with no streptomycin; II, colony counts on streptomycin nutrient agar (1,000 $\mu\text{g/ml}$) of cells incubated with no streptomycin; III, colony counts on streptomycin free nutrient agar of cells incubated with 100 $\mu\text{g/ml}$ of streptomycin; IV, colony counts on streptomycin nutrient agar (100 $\mu\text{g/ml}$) of cells incubated with 100 $\mu\text{g/ml}$ of streptomycin.

majority of the cells again became streptomycin indifferent, and these indifferent cells were all found to have exactly the same markers as those of the indifferent mutants which were mixed with the sensitive cells in the initial inocula. Entirely identical results also were obtained when K 7-1 was marked with T 1 resistance and streptomycin indifference, and mixed with sensitive K 6-70 strain (Experiment 2 in table 3).

Kinetic analysis of "resting cell" experiments. Both Akiba and Szybalski checked the viable cells and streptomycin resistant cells only before and after the incubation of "resting cells" for about two weeks at 37 C and they concluded that either a part or a majority of the cells was "converted" to resistance. As shown above, however, both killing of sensitive cells and multiplication of resistant cells occur under conditions that had been assumed to represent a "resting state." Therefore, it was thought desirable to assay the fate of these "resting cells" from day to day.

The results obtained are shown in figure 1, which indicates that the resistant cells recovered at the end of the incubation with streptomycin are not derived by direct conversion of sensitive cells, but that they originate from the multiplication of a few cells, presumably by utilizing nutrients released from killed sensitive cells. It is

quite conceivable that line III in figure 1 would drop to zero, if the assay were done between the 24 and 48 hr periods, since it has been shown in the other experiments that all sensitive cells are killed very quickly in the "resting state" by streptomycin.

Mutagenicity experiments. The results of the fluctuation tests carried out in order to detect the mutagenic effects of streptomycin on *S. enteritidis* strain 11 are shown in tables 4 and 5. The average mutation rate to streptomycin indifference of this organism was 2×10^{-11} in the absence of streptomycin, and 0.9×10^{-11} in the presence of 0.5 $\mu\text{g/ml}$ of the antibiotic. Accordingly, it could be concluded that at least this

TABLE 4
Mutation rate of *Salmonella enteritidis* strain 11 to streptomycin indifference in the absence of streptomycin

Experiment	No. of Cultures	Vol. of Cultures	No. of Cells	P(0)	Mutation Rate
		ml	$n \times 10^8/\text{ml}$		$a \times 10^{-11}$
A	100	1	5.3	0.96	5.4
B	100	1	8.7	0.96	3.3
C	100	2	18	0.96	1.6
D	72	1	5.7	0.97	4.6
E	100	1	7.9	0.97	3.4
F	100	1	11	0.98	1.3
G	100	2	12	0.97	2.1
H	100	2	22	1.00	0.0
I	100	2	3.4	0.91	4.9
J	100	2	13	0.99	0.6

Average mutation rate: 2×10^{-11} per bacterium per generation.

TABLE 5
Mutation rate of *Salmonella enteritidis* strain 11 to streptomycin-indifference in the presence of 0.5 $\mu\text{g/ml}$ of streptomycin

Experiment	No. of Cultures	Vol. of Cultures	No. of Cells	P(0)	Mutation Rate
		ml	$n \times 10^8/\text{ml}$		$a \times 10^{-11}$
A	100	2	6.5	1.00	0.0
B	100	2	8.5	0.99	1.1
C	100	2	9.4	0.99	0.8
D	100	2	11	0.98	1.2
E	100	2	11	0.98	1.2
F	100	2	8.0	0.99	1.2
G	100	2	10	0.98	0.9

Average mutation rate: 0.9×10^{-11} per bacterium per generation.

concentration of streptomycin has no mutagenic effect on this organism. One may ask if this antibiotic has an antimutagenic action (Novick and Szilard, 1952), but the difference found proved insignificant by T test.

The concentration of 0.5 $\mu\text{g/ml}$ of streptomycin was used because the average resistance of the parent strain, that is, the resistance which most individuals of the population show, was found to be about 1 $\mu\text{g/ml}$. Therefore, most cells of the population should be able to multiply in the presence of 0.5 $\mu\text{g/ml}$ of streptomycin.

DISCUSSION

The foregoing observations again have failed to prove a direct induction of streptomycin resistance in bacteria by the antibiotic itself.

The studies of Akiba and Szybalski have been reinvestigated and their report of the change to streptomycin resistance of either a part or a majority of cells in a "resting" population now must be attributed to a failure to assay viable and resistant cells more frequently than merely before and after a 2-week period of incubation of supposedly "resting cells." A number of experiments by the present authors have demonstrated that thoroughly washed and starved cells of *E. coli* strain K-12, when kept at 37 C in a resting state, are rapidly killed by streptomycin in concentrations higher than 5 $\mu\text{g/ml}$. The fact that selection does take place in "resting cell" populations was revealed by reconstruction experiments employing a large number of sensitive cells and a relatively small number of streptomycin indifferent cells distinguishable by auxotrophic and phage resistant markers. These reconstruction experiments have shown that when "resting cells" are kept in contact with sufficient amounts of streptomycin all of the surviving cells have the same markers as those of the initially added indifferent cells; in contrast, in streptomycin free control suspensions most of the surviving cells proved to be identical with the initially added sensitive cells. These results can be interpreted as indicating that there are no changes in the ratio between sensitive and indifferent cells in the absence of the selective influence of streptomycin. In addition, it should be mentioned that when "resting cells" of *E. coli* strain K-12 were kept at low temperatures (preventing multiplication of the organisms) no increase in streptomycin resistance was obtained with any

combination of bacterial numbers, drug concentrations and duration of incubation.

After the above described experiments had been completed, it was found that Welsch (1951) had reached similar conclusions on the basis of somewhat different types of experiments.

In the present studies the mutagenic effects of streptomycin also were reexamined by determining mutation rates of *S. enteritidis* strain 11 to streptomycin indifference. Similar studies had been reported by Newcombe and McGregor (1951), who concluded that this antibiotic is a weak, but nonspecific mutagen. However as discussed in previous papers, their recognition of a "full resistance" class was based on the phenotype only and thus may have included both the present authors' class of streptomycin indifference and that of high streptomycin resistance. Therefore, in the present studies, cells with an average resistance as low as 1 $\mu\text{g/ml}$ were used and the concentration of streptomycin employed for treatment was only 0.5 $\mu\text{g/ml}$ in order to prevent the participation of selectively developed multistep streptomycin resistant cells. Moreover, the drug concentration used for the recovery of streptomycin indifferent mutants was high: 5,000 $\mu\text{g/ml}$. The experiments carried out with *S. enteritidis* strain 11 under these conditions indicated that 0.5 $\mu\text{g/ml}$ of streptomycin produces no mutagenic effects.

Much of the evidence so far presented for any direct induction of streptomycin resistance by the drug itself seems to have been discredited. However, since many chemicals may act as mutagens when used in proper concentrations, it would not be surprising if some mutagenic action of streptomycin were found in the future. On the basis of presently available information it must be concluded that direct induction of streptomycin resistance, if any, plays no major role in its development in bacteria.

SUMMARY

The effects of streptomycin on the development of streptomycin resistance of washed and starved cells were investigated with *Escherichia coli* strain K-12. It was found that cells suspended in phosphate buffer (so-called "resting cells") actually die or multiply and that the concurrent development of streptomycin resistance in the presence of the antibiotic is due to spontaneous mutation and selection.

No mutagenic effects of streptomycin on the mutation of *Salmonella enteritidis* strain 11 to streptomycin indifference (high one step resistance) could be detected.

REFERENCES

- ABRAHAM, E. P. 1953 The development of drug resistance in microorganisms. In *Adaptation in Microorganisms*. University Press, Cambridge, 21-45.
- ADELBERG, E. A. AND MYERS, J. W. 1953 Modification of penicillin technique for selection of auxotrophic bacteria. *J. Bacteriol.*, **65**, 348-353.
- AKIBA, T. 1952 Bacterial resistance in chemotherapy. *Progress in Medicine*, **13**, 250-271 (in Japanese).
- AKIBA, T. 1953a Development of drug resistance in bacteria and induced mutation. In *New Field of Bacteriology*. Igakushoin Press, 204-221 (in Japanese).
- AKIBA, T. 1953b On the mechanism of the acquisition of drug resistance and its inhibition. *Chemotherapy*, **1**, 1-11 (in Japanese).
- DEMEREK, M. 1948 Origin of bacterial resistance to antibiotics. *J. Bacteriol.*, **56**, 63-74.
- EAGLE, H., FLEISCHMAN, R., AND LEVY, M. 1952 Development of increased bacterial resistance to antibiotics. I. Continuous spectrum of resistance to penicillin, chloramphenicol, and streptomycin. *J. Bacteriol.*, **63**, 623-638.
- EAGLE, H. AND SAZ, A. K. 1955 Antibiotics. *Ann. Rev. Microbiol.*, **9**, 173-226.
- HAYES, W. 1953 The mechanism of genetic recombination in *Escherichia coli*. Cold Spring Harbor Symposia Quant. Biol., **18**, 75-93.
- HINSHELWOOD, C. N. 1949 *Symposia of the Society for Experimental Biology* No. III. Academic Press, New York, 243-252.
- KANAI, K., NAKAMOTO, T., AND YANAGISAWA, K. 1953 In vitro experiments of development of the streptomycin resistance in different strains of *Mycobacterium tuberculosis*. *Japan. J. Med. Sci. Biol.*, **6**, 365-370 (in English).
- LEDERBERG, J., CAVALLI, L. L., AND LEDERBERG, E. M. 1952 Sex compatibility. *Genetics*, **37**, 720.
- LEDERBERG, J. AND LEDERBERG, E. M. 1952 Replica plating and indirect selection of bacterial mutants. *J. Bacteriol.*, **63**, 399-406.
- LINZ, R. 1950 Sur le mécanisme de l'action de la streptomycine; la résistance à la streptomycine. *Ann. inst. Pasteur*, **78**, 105-114.
- LURIA, S. E. AND DELBRÜCK, M. 1943 Mutations of bacteria from virus sensitivity to virus resistance. *Genetics*, **28**, 491-511.
- MITCHISON, D. A. 1953 The occurrence of independent mutations to different types of streptomycin resistance in *Bacterium coli*. *J. Gen. Microbiol.*, **8**, 168-185.
- NEWCOMBE, H. B. 1948 Delayed phenotypic expression of spontaneous mutations in *Escherichia coli*. *Genetics*, **33**, 447-476.
- NEWCOMBE, H. B., AND MCGREGOR, J. 1951 On the nonadaptive nature of change to full streptomycin resistance in *Escherichia coli*. *J. Bacteriol.*, **62**, 539-544.
- NOVICK, A. AND SZILARD, L. 1952 Antimutagens. *Nature*, **170**, 926-927.
- SEVAG, M. G. 1946 Enzyme problems in relation to chemotherapy, "adaptation," mutations, resistance, and immunity. *Advances in Enzymol.*, **6**, 33-127.
- SZYBALKSI, W. 1954 "Lamarckian" inheritance of streptomycin resistance? *Antibiotics Annual*, 1954-55, Medical Encyclopedia Inc., New York, 174-177.
- USHIBA, D. AND WATANABE, T. 1954 Mechanism of increase and decrease of streptomycin resistance of bacteria. I. Effect of streptomycin on resting cells of *Salmonella enteritidis*. *Japanese J. Bacteriol.*, **9**, 349-353 (in Japanese).
- WATANABE, T. 1954a Mechanism of increase and decrease of streptomycin resistance of bacteria. II. Analytical studies on various types of mutations concerning streptomycin. *Japanese J. Bacteriol.*, **10**, 231-237 (in Japanese).
- WATANABE, T. 1954b Genetic studies on the mechanism of acquired streptomycin-resistance in microorganisms. *Keio J. Med.*, **3**, 193-212 (in English).
- WELSCH, M. 1951 Mécanisme de l'apparition de résistance microbienne à la streptomycine après action de l'antibiotique sur des suspensions de bactéries non proliférantes. *Rev. belge pathol. et med.*, **21**, 176-193.
- YOKOTA, T. 1955a On the mechanism of drug resistance. I. The mutagenic effect of streptomycin (1). *Japanese J. Bacteriol.*, **10**, 261-267 (in Japanese).
- YOKOTA, T. 1955b On the mechanism of drug resistance. II. The mutagenic effect of streptomycin (2). *Japanese J. Bacteriol.*, **10**, 313-319 (in Japanese).
- YOKOTA, T. 1955c On the mechanism of drug resistance. III. The mutagenic effect of streptomycin, penicillin and chloramphenicol. *Japanese J. Bacteriol.*, **10**, 418-425 (in Japanese).

YOKOTA, T. 1955*d* On the mechanism of drug resistance. IV. The development of drug resistance in media containing constant doses of streptomycin, penicillin and chloramphenicol. Japanese J. Bacteriol., **10**, 509-514 (in Japanese).

YOKOTA, T. 1955*e* On the mechanism of drug resistance. V. On the specificity of mutagenic effect of streptomycin. Japanese J. Bacteriol., **10**, 651-656 (in Japanese).

ZELLE, M. R. 1955 Genetics of Microorganisms. Ann. Rev. Microbiol., **9**, 45-98.

ERRATA

THE MICROBIOLOGY OF COAL

I. BACTERIAL OXIDATION OF PHENANTHRENE

MARTIN H. ROGOFF AND IRVING WENDER

Bureau of Mines, U. S. Department of the Interior, Region V, Bruceton, Pennsylvania

In the above article, which appeared in the *Journal* (J. Bacteriol., **73**, 264-268), the first paragraph should be deleted.

EXCRETION BY STREPTOMYCETES OF FACTORS CAUSING FORMATION OF AERIAL HYPHAE BY OLD CULTURES

NORMAN C. DONDERO AND TULLIO SCOTTI

Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey

An error appeared in the above article published in the April issue of the *Journal* (J. Bacteriol., **73**, 584-585, 1957). In table 1, the column headings are reversed. Heading for column 1 should read "Stimulating Strains"; heading for column 2 should read "Stimulated Strains."