tween *Escherichia coli, Pseudomonas aeruginosa,* and *Aerobacter cloacae*—they were sufficiently consistent to differentiate the species studied to date. Infrared spectra do not always group species as in the Bergey classification. For example, *Pseudomonas* (Family II) organisms and *Escherichia* (Family X) have similar spectra, whereas *Micrococcus rosaceous* and *M. pyogenes* var. *aureus* (Genus I, Family V) have very different spectra.

In addition to differentiation of species, it was possible to differentiate strains of *Bacterium tularense*

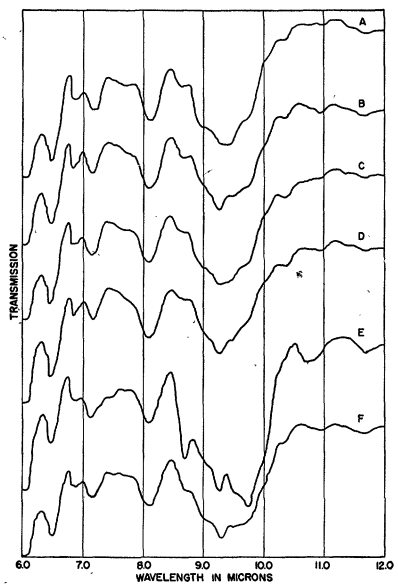

FIG. 3. Spectra of various strains of *Bacterium tularense* cultured for 24 hr on tryptose agar: (*A*) *Schu* S_3; (*B*) *Schu* NS_3; (*C*) Jap S_2; (*D*) Jap S_4^{ns}; (*E*) 38 NS_2; (*F*) 38 S_1.

(Fig. 3). Spectroscopic differentiation of the *Schu* S_3 smooth strain and the Jap S_2 smooth strain (*8*) is somewhat uncertain, but the differences between other strains studied are quite obvious. Spectra of all strains of *B. tularense* are characterized by a sudden drop in transmission at 6.80 μ, producing a clearly marked minimum at that point. The spectra of all other organisms thus far observed have a minimum transmission at 6.90 instead of 6.80 μ.

The use of infrared absorption spectra as an aid to identification appears promising. The prerequisite for such identification is a catalogue of spectra of organisms cultured under controlled conditions.

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Effect of Penicillin on Streptomycin-dependent Variants in *Escherichia coli* Populations

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The penicillin method for the isolation of biochemically deficient mutants (*1–3*) has been applied to the problem of the origin of streptomycin-dependent variants of *Escherichia coli.* If dependent cells arise spontaneously in an actively growing culture of normal bacteria, they should soon stop growing, since no streptomycin is present and since their requirement for this substance has been shown to be highly specific. If this cessation of growth occurs while the remainder of the population is still growing actively, penicillin may be expected to kill the normal cells and spare the streptomycin-dependent ones, as in the usual isolation of biochemically deficient mutants by this method. If, on the other hand, streptomycin-dependence represents an adaptation to the antibiotic, no reason is known why the precursors in the normal population before contact with streptomycin would be uniquely insensitive to the bactericidal action of penicillin.

A smooth, motile strain of *E. coli* was used, which behaved typically in the usual series of characterization tests. The organism was grown from small inocula at 37° C in Difco nutrient broth with 0.2% added glucose. The inoculum size was estimated by replicate platings of identical inocula on nutrient agar. Growth of the broth culture was followed by turbidimetry and viable counts. When the desired degree of growth had been reached (usually $1–3 \times 10^8$ cells/ml), the culture was centrifuged and resuspended in fresh nutrient broth with glucose. A viable count was made, and 0.3 ml implanted onto each of 6 nutrient agar plates containing streptomycin (*SM*), 10 μg/ml. These plates had been previously dried so that the implant fluid would be absorbed within 30 min at room temperature. Penicillin (300 u/ml) was added to the remainder of the resuspended culture at 37° C; the culture was then incubated for 20–40 min and quickly immersed in an ice bath. After centrifuging, the penicillin-containing broth was replaced by the same volume of 0.9% NaCl. Another viable count was done to determine the extent to which penicillin had killed the normal population. A second set of implants on *SM* plates was then made, exactly as before. All the plates were incubated at 37° C.

With this organism, at the *SM* concentration used here, dependent (*D*) colonies appear on the plates with a characteristic delay of 1–4 days. Of thousands of *D* colonies observed in numerous experiments, none has ever been macroscopically visible 24 hr after implantation. Nondependent, resistant (*R*) colonies, on the other hand, almost always are present at 24 hr,

TABLE 1

STREPTOMYCIN-RESISTANT AND STREPTOMYCIN-DEPENDENT COLONIES APPEARING ON STREPTOMYCIN PLATES IMPLANTED WITH ALIQUOTS OF A NORMAL *E. coli* CULTURE, BEFORE AND AFTER EXPOSURE TO PENICILLIN*

SM plate	Days after implantation					Total
	1	2	3	4	5	
Before penicillin						
1	2 *R*	11 *D*	5 *D*	3 *D*	None	2 *R*, 19 *D*
2	3 *R*	7 *D*	10 *D*	2 *D*	"	3 *R*, 19 *D*
3	5 *R*	5 *D*	7 *D*	1 *D*	"	5 *R*, 13 *D*
4	4 *R*	12 *D*	11 *D*	1 *D*	"	4 *R*, 24 *D*
5	6 *R*	2 *R*, 11 *D*	6 *D*	2 *D*	2 *D*	8 *R*, 21 *D*
6	2 *R*	8 *D*	6 *D*	None	None	2 *R*, 14 *D*
Total	22 *R*	2 *R*, 54 *D*	45 *D*	9 *D*	2 *D*	24 *R*, 110 *D*
After penicillin†						
7	None	None	1 *D*	None	None	1 *D*
8	"	"	2 *D*	"	"	2 *D*
9	"	1 *D*	3 *D*	"	"	4 *D*
10	"	1 *D*	1 *D*	"	"	2 *D*
11	"	2 *D*	2 *D*	"	"	4 *D*
12	"	3 *D*	1 *D*	"	"	4 *D*
Total	None	7 *D*	10 *D*	None	None	0 *R*, 17 *D*

* Broth culture of *E. coli*: Difco nutrient broth with 0.2% glucose, inoculated with 600 cells, all sensitive to *SM*. Experiment run after 16¾ hr growth, in the phase of growth deceleration. Implants onto streptomycin plates (10 μg *SM*/ml): onto each plate 0.3 ml containing, before penicillin, 1.59×10^8 viable cells. Same implant volume was used after exposure to penicillin. Exposure to penicillin: 300 u/ml for 40 min at 37° C in fresh nutrient broth with 0.2% glucose.

† Viable after exposure to penicillin, as percentage of initial viable count, 0.120%; dependent colonies after exposure to penicillin, as percentage of initial number, 15.4%; probability that this result is due to chance—i.e., random sampling error with no differential killing by penicillin—$P \ll 0.001$.

although occasionally not until the following day. (The unusual lag in the initial growth of *D* colonies was first observed by Yegian, Budd, and Vanderlinde (*4*) with *Mycobacterium tuberculosis*.) When the delayed *D* colonies are subcultured on *SM* agar, discrete colonies usually grow without delay, in 24 hr. Further studies of the lag phenomenon will be reported elsewhere.

All plates were therefore incubated for 5 days, after which time no new colonies have ever been observed to appear. To avoid any possibility of accidental seeding of secondary colonies, plates were marked daily but not opened until the fifth day. At that time every colony was tested for ability to grow on nutrient agar, with and without *SM*. Heavy growth was always obtained on *SM* agar. Classification as *R* or *D* was made on the basis of growth on agar without *SM*.

The results of a typical experiment are shown in Table 1. An actively growing culture in the phase of growth deceleration was used. The mean generation time, measured during the final hour of growth, was 3.0 hr. The data presented in Table 1 show unequivocally that the bactericidal action of penicillin differentiates between normal and *D* cells. Whereas only 0.120% of the whole population survived exposure to penicillin, the corresponding figure for *D* cells was 15.4%. Thus, after penicillin, 128 times as many *D* colonies appeared as would be expected on the basis of the total viable count. If the precursors of *D* colonies were as sensitive to the bactericidal effect of penicillin as is the rest of the population, the mean expected number of *D* colonies after penicillin should be 0.120% of the number present before penicillin, or 0.132 colonies. From the Poisson distribution it can be calculated that in 87.6% of similar experiments no *D* colonies should remain after penicillin but in 11.6% a single *D* colony should be found. The probability of the observed result (17 colonies) having occurred as a result of random sampling without differential killing by penicillin is infinitesimal (less than once in 10^{29} such experiments).

In other experiments the relative killing of normal and *D* cells varied, partly as a function of the growth phase of the culture used, and partly in response to other factors not yet analyzed. In a culture that had practically ceased growth in the early stationary phase, the number of *D* colonies after penicillin was 94% of that present initially, and 37% of the whole population survived exposure to penicillin. When a culture was left in contact with penicillin for longer than 3 hr (as in the routine isolation of biochemical mutants), the entire population was practically sterilized, and no *D* cells survived. The immunity of *D* cells to the bactericidal action of penicillin is thus not absolute, but they are evidently killed more slowly than the rest of the population. This is consistent with the finding of Schaeffer (*5*) and with the author's observations that *D* cells are capable of a limited amount of slow growth in the absence of *SM*.

The complete elimination of the *R* colonies shown in Table 1 was to be expected, but no general infer-

ence can be drawn because of the small number initially present. In numerous other experiments, however, where killing by penicillin was less extreme, the number found after exposure to penicillin was reduced to about the same extent as the total viable count. This is reasonable in view of the equivalent growth rates of normal and *R* cells in nutrient broth.

The disproportionate survival of *D* variants when the total viable count is reduced by penicillin could conceivably be an artifact if for any reason the number of *D* colonies appearing in implants from a given culture was not proportional to the number of viable cells in the implants, when no penicillin had been used. Peculiar effects of this kind were noted by Barer (*6*). To rule out such a possibility, control experiments were performed in which diluted (1 : 20) and undiluted implants from the same culture were plated onto *SM* agar. The number of *D* (and *R*) colonies from the diluted implants was consistently about 5% of the number from undiluted implants. This shows that when no differential bactericidal effect is operative, the number of *D* colonies obtained from a given culture is approximately proportional to the viable count of the implant.

To show that the original small inoculum used contained no cells capable of growth on *SM*, several implants of more than 300,000 cells were made on *SM* agar. As these yielded no growth whatever, it can be confidently stated ($P < 0.002$) that the inoculum of 600 cells in the experiment of Table 1 contained no *R* or *D* cells, and that both these variants must have arisen during growth of the broth culture.

The findings reported here provide clear-cut evidence that a normal bacterial population is inhomogeneous from the standpoint of the ability to give rise to *SM*-dependent colonies on implantation onto *SM* agar. The number of *D* colonies appearing is approximately proportional to the total viable count of the implant under normal conditions, *but not when the culture has been exposed to penicillin.* After such exposure the number of *D* colonies decreases much less than the total viable count. The simplest and most satisfactory explanation of the inhomogeneity, consistent with what is known about the preferential action of penicillin upon actively growing cells, is that *SM*-dependent organisms are themselves present in a normal population before contact with *SM*. Such cells may be presumed to arise by spontaneous mutation, and may be thought of as lethal mutants which will only survive and multiply if transferred to a *SM*-containing medium. Their relative insensitivity to penicillin would result from their poor growth in the absence of the specific growth requirement, and the position would be analogous to that of the various biochemically deficient mutants. The only alternative explanation would be that precursors of *D* cells are present in a normal population, that these are relatively insensitive to penicillin, and that they only give rise to *SM*-dependent clones by some adaptive process after transfer to a *SM* medium. If slow growth were especially favorable to adaptation, the *D* precursors might be those cells in the normal population that grow most slowly and are therefore also relatively insensitive to penicillin. Experiments now in progress are designed to distinguish between these two alternatives.

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Cocontraction and Reciprocal Innervation in Voluntary Movement in Man

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The coordination of muscular activity in voluntary movement involves a number of neuromuscular phenomena, one of the most important of which is the alternate action of antagonistic muscles. It has been a seemingly empirical observation known to many that antagonists cease to function when agonist muscles begin to contract. A simple example of this is the relationship of the triceps and the biceps in alternate flexion and extension of the elbow. When the elbow is flexed, the triceps is relatively inactive; when the elbow is extended, the biceps is quiet. There is no doubting the observation. However, there has been a gross oversimplification of the nature of its causation and its actual occurrence under all conditions of muscular contraction.

The most commonly accepted thesis in regard to the interrelationship of antagonistic muscles states that the contraction of a muscle produces by proprioceptive action a central inhibitory effect on its antagonist muscle. This hypothesis is based on the huge body of excellent work reported by Sherrington (*1*) in a series of classic papers. The inhibitory effect upon the skeletal muscles is not brought about through specific inhibitory nerves such as the vagus when it causes cardiac inhibition or the sympathetic nerves which cause inhibition of contraction of the intestinal muscles. Direct stimulation of motor nerves to the skeletal muscles results only in excitation. The inhibitory process is, therefore, considered to be central in origin. Stimulation of proprioceptive end organs in the contracting muscle is thought to cause cessation or diminution of excitatory impulses along the motoneuron to the antagonist muscle.

The alternate inhibition and stimulation of contraction in antagonistic muscles was labeled by Sherrington as reciprocal innervation. Innumerable experiments performed by him and by later observers substantiated the existence of this phenomenon. However, most of the observations were made with decerebrate or spinal animals or animals under anesthesia, in all