presupposes the existence of a highly complex chemical environment.

To summarize, the hypothesis presented here suggests that the first living entity was a completely heterotropic unit, reproducing itself at the expense of prefabricated organic molecules in its environment. A depletion of the environment resulted until a point was reached where the supply of specific substrates limited further multiplication. By a process of mutation a means was eventually discovered for utilizing other available substances. With this event the evolution of biosyntheses began. The conditions necessary for the operation of the mechanism ceased to exist with the ultimate destruction of the organic environment. Further evolution was probably based on the chance combination of genes, resulting to a large extent in the development of short reaction chains utilizing substances whose synthesis had been previously acquired.

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*STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. V. STRAIN RESISTANCE OF BACTERIA TO ANTIBIOTIC SUBSTANCES, ESPECIALLY TO STREPTOMYCIN**

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Different strains of the same species of bacteria are found to vary greatly in their sensitivity to a given antibiotic substance. This phenomenon has an important bearing upon the utilization of the substance for chemotherapeutic purposes, where a knowledge of the sensitivity of the particular strain of a given organism responsible for a certain disease becomes of paramount importance.

It has been definitely established that when a culture of an organism is grown in the presence of a certain antibiotic substance, it becomes resistant to increasing concentrations of the substance; strains may thus be obtained that have become adapted to the action of the antibiotic agent and that require far greater concentrations for growth inhibition as compared with the parent or mother culture from which they were isolated. This has been shown to hold true for the action of lysozyme on *Micrococcus lysodeikticus*, and for various antibiotic and chemical agents against different bacteria. This phenomenon has been studied extensively in recent years as applied to the effect of penicillin on a number of bacteria, including *Staphylococcus aureus*,[1] pneumococci,[10] staphylococci, streptococci,[9] gonococci[2] and various others. Different strains of *S. aureus* have been shown[11] to vary in their susceptibility to penicillin in a range of less than 0.1 unit to greater than 1.0 unit.

Davies, Hinshelwood and Price[3] explained the phenomenon of adaptation of an organism to an antibacterial agent by one or more of the following mechanisms: (1) Adaptation occurs by natural selection from an initially heterogeneous population; this concept has lost, however, much support, since variations have been found to occur in strains derived initially from a single cell. (2) Adaptation occurs by actual modification of the individual cells; this may be due to the establishment in the cells of a mechanism alternative to that normally in use, or a quantitative modification of the existing mechanism occurs. (3) Adaptation is a change in some center of organization of the cell. In summarizing the various theories explaining the phenomenon of bacterial adaptation, Hinshelwood[7] concluded that when variations or adaptive changes occur, there is an actual modification of the character of the individual cells, although selection may be superimposed on this when modified and unmodified cells exist together. Demerec[4] concluded that resistance of *S. aureus* to penicillin originates through a mutation mechanism, and that penicillin acts as a selective agent to eliminate the non-resistant members of the bacterial population.

The adaptation of a certain bacterial strain to a given antibiotic substance does not necessarily signify that the strain is also resistant to another substance. A certain organism may become more resistant to penicillin by growth in gradually increasing concentrations of this antibiotic in an artificial culture medium, or more resistant strains of this organism may be isolated from patients treated with penicillin. Such strains do not necessarily show the same range of resistance to another antibiotic substance, like streptomycin. It was found,[15] for example, in a study of the variation of spore-forming bacteria, that different strains resistant to streptothricin are not at all resistant to clavacin or to fumigacin. Different strains of staphylococci were found[11] to show different degrees of resistance to penicillin, tyrothricin and streptothricin, the action of one antibiotic not being necessarily paralleled by the action of another. When resistant strains are grown in media free from the antibiotic, a reversion may occur either gradually or suddenly and spontaneously; however, some of the resistant variants may be very stable.[7] The sensitivity of different strains of the same organism to a single antibiotic may also depend upon the morphological and physiological characteristics of the strains, as shown for the sensitivity of sporulating and non-sporulating strains of *Actinomyces griseus* to streptomycin.[14]

In the following investigations a study has been made of the sensitivity and adaptation of different strains of various gram-negative and gram-positive bacteria to streptomycin.[12] This antibiotic agent was selected because of its specific capacity to inhibit the growth of bacteria belonging to both gram-negative and gram-positive groups,[6, 8, 13] and because of its chemotherapeutic potentialities.[8]

TABLE 1

EFFECT OF STREPTOMYCIN UPON DIFFERENT STRAINS OF *E. coli* AND *E. communior*

E. coli		*E. communior*	
CULTURE NO.	D. U.[a]	CULTURE NO.	D. U.
ATCC 26	30	ATCC 130	30
ATCC 6522	30	ATCC 4163	30
ATCC 6880	10	ATCC 4351	30
ATCC 6881	30	ATCC 4352	30
ATCC 8677	100	ATCC 7011	30
ATCC 8739	30	Average	30
W 2	30		
W 4	30		
W 5	30		
Average	35		

[a] D. U. = dilution units per 1 mg. as determined by plate method.

Experimental.—Most of the cultures of bacteria used in these studies were obtained from the American Type Culture Collection (ATCC), though some were taken from our own collection (W), and some were received from special sources, as will be indicated. The streptomycin used in most of the experiments was a dry, fairly stable preparation produced in our own laboratory; it had an activity of 58 units per milligram.

In the first group of experiments, a number of pure cultures of bacteria comprising three gram-negative and two gram-positive species were tested for their sensitivity to streptomycin. The results presented in table 1 show that out of nine strains of *Escherichia coli* and five strains of *E. communior*, only one was very sensitive (100 units) to the antibiotic agent and one was very resistant (10 units); the remaining twelve strains were about equally sensitive, giving about 30 units as measured by the agar streak method.

Greater variation was obtained in the sensitivity to streptomycin of different strains of *Proteus vulgaris* and *S. aureus* tested by the same method, although the average values for these organisms as well were about the same as for *E. coli* (table 2). Out of seven cultures of *Pr. vulgaris*

TABLE 2

EFFECT OF STREPTOMYCIN UPON *S. aureus* AND *Pr. vulgaris*

Pr. vulgaris		*S. aureus*	
CULTURE NO.	D. U.	CULTURE NO.	D. U.
ATCC 6898[a]	30	ATCC 152	25
ATCC 7829	75	ATCC 6518	25
ATCC 8259	50	ATCC 6538	45
ATCC 8427[a]	10	ATCC 8094	30
ATCC 9484	30	ATCC 8431	20
W 1[a]	30	W 1	30
W 2[a]	20	W 2	25
Average	35	W 4	75
		Merck Institute	30
		Average	34

[a] W 1 is same strain as 6898; W 2 is same as 8427.

used in these tests, one was very resistant (10 units) and one was rather sensitive (75 units), the remaining five cultures showing sensitivity to the particular streptomycin preparation ranging from 20 to 50 units, with an average of 35 units. The range in sensitivity of the nine *S. aureus* strains was from 20 to 75 units, with an average of 34 units.

The sensitivity of six strains of *Bacillus subtilis* to streptomycin is brought out in table 3. In the case of this organism, as well, there was considerable

TABLE 3

EFFECT OF STREPTOMYCIN UPON DIFFERENT STRAINS OF *B. subtilis*

DILUTION (PLATE) METHOD		DIFFUSION (CUP) METHOD. ZONE OF INHIBITION, MM.	
CULTURE	D. U.	DILUTION 1:5	DILUTION 1:25
ATCC 102	30	19.5	13.0
N. R. Smith 237	100	19.0	14.5
N. R. Smith 972	30	16.8	12.0
ATCC 6633[a]	250	26.3	20.5
ATCC 8473	100	23.0	17.5
W 9[a]	250	26.0	20.0
Average	109		

[a] ATCC 6633 is the same strain as W 9.

variation among the different strains. The tests with this organism were carried out by two methods, namely, the agar streak or plate dilution and the agar diffusion or cup methods. The degree of sensitivity of *B. subtilis* to streptomycin was found to be characteristic of the strain rather than of the organism. Two strains, 102 and 972, were markedly resistant to streptomycin, as measured by both methods, the degree of resistance being of about the same order of magnitude as for the average strain of *E. coli.* Most of the strains of *B. subtilis* were, however, much more sensitive. The most sensitive strain was available in two different cultures, namely, ATCC 6633 and W 9; both showed the same degree of sensitivity by both methods of testing. Strain 237 produced by the cup method, in addition to the clear, easily readable zones, also secondary and even tertiary zones, which tended to confuse the readings, since only the clear zones were measured Finally, strain 8473 did not produce the same type of growth as did the other strains of *B. subtilis;* it formed rough surface colonies which gave the plates a peculiar appearance, although they did not interfere with the reading of the zone of inhibition. With the exception, therefore, of the last two cultures, the strains of *B. subtilis* varied in their sensitivity to streptomycin as much as 10:1.

TABLE 4

BACTERICIDAL EFFECT OF STREPTOMYCIN UPON *Pr. vulgaris*

INCUBATION WITH STREPTOMYCIN, HRS.	STREPTOMYCIN PER 1 ML. CULTURE			
	0	2	10	50
	MILLIONS OF CELLS PER 1 ML.			
0	1,290	1,290	1,290	1,290
6	2,150	1,010	665	100
24	1,470	110	30	2.3
48	745	1.2	0[a]	0[a]
124	360	0	0	0

[a] 1–4 colonies on a plate from 1:100,000 dilution.

In order to establish the manner in which strains of a given culture are capable of developing resistance against streptomycin, *Pr. vulgaris* was selected for more detailed studies. At first, the effect of the concentration of streptomycin upon its bactericidal action was determined by using a single strain (No. 2) of *Pr. vulgaris.* The results of a typical experiment are given in table 4. When 2.0 to 10 units of streptomycin were added to a culture of *Pr. vulgaris* all the cells were killed in 48 to 124 hours; when one unit was used, there was only a temporary inhibition of growth, which was later overcome. Cultures of *Pr. vulgaris,* treated with the larger amounts of streptomycin, were streaked, after 6 days' incubation, on nutrient agar plates, and the colonies produced from a few surviving cells were isolated and transferred to fresh media. A new strain was thus obtained possessing all the cultural and staining properties of the mother culture; however, it proved to be more resistant to streptomycin. This new strain was designated as R. Two original strains and the resistant form were inoculated into several lots of fresh broth containing varying concentrations of streptomycin. The numbers of viable bacteria were determined after several periods of incubation at 37°C. The results reported in table 5 show emphatically that strain R, isolated from culture No. 2, became highly resistant to the action of streptomycin.

The sensitivity of the original culture of *Pr. vulgaris* and that of the resistant strain R was now measured against streptothricin, an antibiotic substance closely related to streptomycin. The results, which need not be reported here in detail, tended to demonstrate that strain R, made resistant to streptomycin, also became, though perhaps not to so great a degree, resistant to streptothricin, thus pointing to a certain similarity in the two substances. When, however, a totally different type of antibiotic agent was used, namely, clavacin, no difference could be found in the sensitivity of the streptomycin-resistant strain R as compared to that of

TABLE 5

BACTERICIDAL ACTION OF STREPTOMYCIN UPON *Pr. vulgaris* AND A FRESHLY ISOLATED RESISTANT STRAIN OF THIS ORGANISM

Number of Cells in Millions per 1 Ml.

STREPTOMYCIN UNITS PER 1 ML.	CULTURE W 1	CULTURE W 2	STRAIN R
	AT START		
	39.5	45.5	26.0
	AFTER 28 HRS.' INCUBATION		
0	375.0	615.0	560.0
1	0.01	0.65	190.0
5	0[a]	0[a,b]	9.0
25	0	0[a,b]	3.13

[a] 1 colony on a plate from 1:1000 dilution.
[b] 7–8 colonies on a plate from 1:10 dilution.

the mother culture No. 2. Thus, a strain of a bacterium that becomes resistant to a given antibiotic substance may also be resistant to a related compound, but not to a different type of antibiotic substance.

The results of a more detailed study of this phenomenon, using the mother culture and the resistant strain R of *Pr. vulgaris*, as well as two corresponding strains of *S. aureus*, are presented in table 6. With the first organism, the previous observations are confirmed. With *S. aureus*, however, the strain made more resistant to streptomycin was not rendered resistant to streptothricin. A possible explanation for this discrepancy may be found in the fact that the resistance of *S. aureus* to streptomycin was not increased sufficiently. The ratio of the activity of streptomycin against the parent strain of *Pr. vulgaris* to that against the resistant strain is about 12:1, whereas the ratio of the activities of streptothricin against these two strains is 6:1. With *S. aureus*, however, streptomycin was only five times less active against the resistant strain than against the parent strain. Thus, if one assumes that these two agents act similarly against *Pr. vulgaris* and *S. aureus*, the resistance to streptomycin of the new strain of *S. aureus* is not sufficiently higher than that of the parent strain for differences in their resistances to streptothricin to become apparent.

The action of several antibiotic substances upon four cultures of *S. aureus* and their corresponding strains made resistant to streptomycin was now determined. These cultures were obtained from The Merck Institute of Therapeutic Research. The ratio of sensitivity to streptomycin of the resistant strains to that of the mother cultures varied from 750 to >2000. When tested against streptothricin, the corresponding ratios were 1 to 4, thus indicating only a very low degree of increase in resistance. When tested against clavacin and St, an antibiotic substance

TABLE 6

BACTERIOSTATIC ACTIVITY OF ANTIBIOTIC SUBSTANCES UPON STREPTOMYCIN-RESISTANT STRAINS OF TWO BACTERIA

	ACTIVITY, DILUTION UNITS PER MG.[a]				
	Pr. vulgaris			*S. aureus*	
ANTIBIOTIC AGENT	STRAIN 1	STRAIN 2[b]	RESISTANT STRAIN R	PARENT STRAIN	RESISTANT STRAIN
Streptomycin	250	100	8	250	50
Streptothricin	250	150	25	250	250
Clavacin	150	150	150	200	250

[a] The activity of the antibiotic agents was for streptomycin, 58 units; streptothricin, 500 units; and for the crystalline clavacin, 200 units; the actual results obtained for streptomycin were multiplied by 8.3, namely, the activity ratio of the streptothricin and streptomycin preparations, in order to make them comparable.

[b] Strain 2 is the parent culture from which the resistant strain R has been isolated.

produced by an aerobic spore-forming bacterium, no difference whatsoever in the sensitivity of the original cultures and streptomycin-sensitive strains could be detected.

Summary.—Different strains of the same bacterial species may vary greatly in their sensitivity to streptomycin. The ratio of sensitivity to a given preparation varied for *E. coli* from 100 to 10 units with an average of 35; for *Pr vulgaris,* 75 to 10 units (average 35); for *S. aureus,* 20 to 75 units (average 34); and for *B. subtilis,* 30 to 250 units (average 109).

A streptomycin-resistant strain of *Pr. vulgaris* showed also a certain degree of resistance to streptothricin but none at all to clavacin.

A strain of *S. aureus* that was made only slightly resistant to streptomycin showed no resistance to streptothricin. Several highly resistant strains of *S. aureus* showed no increase in resistance to clavacin and to an antibiotic substance isolated from a spore-forming soil bacterium, and only a trace of increased resistance to streptothricin.

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*STABILIZING INFLUENCE OF LIBERAL INTAKE OF VITAMIN A**

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Recently we have reported evidence that liberality of nutritional intake of vitamin A tends to postpone aging and to increase the length of life.[1]

It is now found further that offspring of the (rat) families having the higher vitamin A intakes grow not only somewhat more rapidly but also with distinctly lesser individual variability, thus indicating both a favorable and a stabilizing influence, of liberal vitamin A, upon the growth process.

Table 1 shows the mean weights at specified ages and the mean gain in weight from the 28th to the 56th days of age (end of infancy to early adolescence in the rat) for strictly parallel series of experimental animals on Diets 16, 360 and 361, which are otherwise alike but contain, respectively, 3, 6 and 12 International Units of vitamin A per gram of air-dry food; or 0.8, 1.6 and 3.2 I. U. per food calorie.

It will be seen that early growth is more rapid on the diets with 6 or 12 than on that with 3 I. U. per gram; while the growth was essentially alike on the two higher levels. This was equally true for each sex. Weights