influenza virus against which they did possess antibodies. If the mechanisms of infection with those 2 viruses are the same, non-specific cellular resistance resulting from infection with one virus should have conferred increased resistance to infection with the antigenically distinct virus. The results, therefore indicate that following an original influenza virus infection either (1) non-specific cellular refractoriness plays little, if any part in the resistance of ferrets to reinfection with that virus or (2) the mechanisms of infection with the A and B viruses are not the same.

It is of interest that when PR8 was used for inoculation (Chart 1), the animals previously infected with Czech showed a higher fever, an earlier peak and a less prolonged febrile reaction than did the normal animals. If that difference in response was not due to chance these results might be interpreted as an indication that the convalescing animals were even more susceptible to infection than were the normal ferrets.

Summary. Ferrets which had been infected with either influenza A or influenza B virus were tested for immunity to those 2 viruses on the eighth day of convalescence. They were susceptible to infection when reinoculated with the heterologous virus against which they possessed no antibodies, but were immune to infection when reinoculated with the homologous virus against which they possessed antibodies. If the mechanisms of infection with the A and B viruses are the same the results indicate that non-specific cellular refractoriness plays little part in the resistance of ferrets to reinfection with influenza virus.

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Observations on the Action of Streptomycin *in vitro* (I).*

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Since the discovery of streptomycin by Waksman,[1] a number of reports on the sensitivity of microorganisms to this antibacterial agent have appeared.[2-8] The sensitivities have been expressed in terms of units per cc or micrograms of streptomycin base per cc necessary for inhibition of growth. The sensitivities reported have varied widely depending upon the individual strain tested. Strains which have been considered sensitive to streptomycin and which have been inhibited in some laboratories by a few tenths of a microgram per cc have in other laboratories required from 1 to 10 μg per cc or more for inhibition. These differences have depended upon the experimental conditions, and upon the criteria of inhibition used.

It was early demonstrated by Waksman,[2,3] Donovick,[9] and others[10,11] that medium influ-

* A part of this work was presented before the Society of American Bacteriologists, May, 1946.

1 Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.

2 Waksman, S. A., Reilly, H. C., and Schatz, A., *Proc. Nat. Acad. Sci.*, 1945, **31**, 157.

3 Waksman, S. A., and Schatz, A., *J. Am. Pharm. Assn.*, Sci. Ed., 1945, **34**, 273.

4 Buggs, C. W., Bronstein, B., Hirschfield, J. W., and Pilling, M. A., *J. Am. Med. Assn.*, 1946, **130**, 64.

5 Helmholz, H. F., *Proc. Staff Meeting, Mayo Clinic*, 1945, **20**, 357.

6 Feldman, W. H., and Hinshaw, H. C., *Am. J. Path.*, 1946, **22**, 640.

7 Alexander, H. E., *J. Pediat.*, 1946, **29**, 192.

8 Keefer, C. H., *J. Am. Med. Assn.*, 1946, **132**, 4, 70.

9 Donovick, R., and Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 224.

10 Bondi, A., Dietz, C. C., and Spaulding, E. H., *J. Bact.*, 1946, **52**, 150.

11 Hobby, G. L., Lenert, T. F., and Hyman, B., *J. Bact.*, 1946, **51**, 606.

ences the sensitivity of an organism to streptomycin. Age and density of culture were recognized as significantly affecting sensitivity.[7,11] The significance of these factors has been further emphasized by the recent work of Berkman and his coworkers.[12]

Despite recognition of the fact that sensitivity could be altered by such factors as these, no standardized procedure for determination of the sensitivity of organisms to this agent has been utilized.

The present study was undertaken in an attempt to determine the range of sensitivity of various bacterial species to streptomycin, using a standardized procedure, and to determine certain of the factors which may influence the apparent sensitivity of an organism to streptomycin.

Method. Except when otherwise specified, a beef infusion medium† buffered at pH 7.8 was used throughout. This medium contained a relatively high concentration of phosphates which may decrease to some extent the activity of streptomycin. However it was felt advisable to use this in view of the fact that it is sufficiently rich to support the growth of practically all pathogenic organisms.

Six-hour plain broth cultures were used except when otherwise indicated. These cultures were prepared from freshly cultivated 16-18 hour broth cultures, and were diluted with broth to a constant density immediately prior to use. A density equivalent to a MacFarland $BaSO_4$ No. 1 standard and allowing 70 to 78% transmission on a Photovolt Lumetron No. 400 was arbitrarily chosen as standard. This density corresponds to a concentration of 2-400 million organisms per cc for the majority of strains tested. The sensitivity of a standard strain of *E. coli* was determined simultaneously with each series of unknown organisms.

For each organism tested, a series of 12 tubes were set up containing 0.01 to 0.1 cc, 0.15 cc, and 0.2 cc of a broth solution containing the equivalent of 100 μg of streptomycin‡ per cc. The total volume of each tube was adjusted to 0.5 cc with sterile broth and 0.5 cc of a 10^{-3} dilution of the standardized culture was then added to each. The final concentration of organisms was therefore about 150,000 per cc; the final concentration of streptomycin varied from 1.0 to 20 μg per cc. In the case of highly sensitive organisms, a solution of streptomycin containing 25 μg per cc was at times necessary whereas for more resistant organisms, a solution containing 1000 to 2000 μg per cc was used. Unless otherwise specified, impure commercial streptomycin sulfate was used. Incubation was carried out at 37°C. for a period of 72 hours. The amount of growth was recorded at 24, 48, and 72 hours. The sensitivity of an organism was accepted as the least amount of streptomycin causing complete inhibition of growth, as evidenced by absence of gross turbidity, after 72 hours' incubation. It was recognized however that this did not necessarily indicate a bactericidal level.

Experimental. Using this procedure experiments were carried out (1) to demonstrate the effect of variation in culture on the sensitivity of an organism to streptomycin, and (2) to demonstrate the effect of medium on the sensitivity of an organism. In addition the sensitivity of 84 freshly isolated strains belonging to 10 different species was tested against impure streptomycin sulfate. Certain of these strains were also tested for compar-

[12] Berkman, S., Henry, R. J., and Housewright, R. D., *J. Bact.*, 1947, in press.

† This medium was first used by Dawson[13] for cultivation of pneumococcus variants. It has since been widely used in a number of laboratories for various purposes including the primary isolation of a wide variety of pathogens. It readily supports growth of many organisms that are often difficult to cultivate in the usual beef infusion mediums. Lean chopped beef (1 lb per liter of water) is allowed to infuse at 5°C for 18 to 24 hours. The mixture is then boiled for 15 minutes, and filtered through cotton cloth. Sodium phosphate (Na_2HPO_4; 4 g/liter) and neopeptone (10 g/liter) are then added, the mixture boiled for 15 minutes, and filtered through filter paper while hot. After adding sufficient water to bring the volume to 1 liter, the pH is adjusted to 8.0 with 2N NaOH and the mixture boiled long enough to clear (no longer). The medium is then tubed and autoclaved at 15 lb pressure for 20 minutes.

‡ All streptomycin used throughout this study was prepared by Chas. Pfizer and Co.

TABLE I.
Bacteriostatic Action of Streptomycin on a Standard Strain of *E. coli in Vitro.*

Streptomycin preparation	Potency μg/mg	Sensitivity in μg/cc* 24 hr	48 hr	72 hr
I	249	4.5	6	6
		7	7	7
II	418	7	8	8
		6	8	8
		7	7	8
		4	4	4
		5	5	5
		5	5	5
		4	4	4
III	802	5	5	5
		5	5	5
		9	9	9

* Sensitivity = Least amount of streptomycin causing complete inhibition of growth.

ison against the crystalline $CaCl_2$ double salt of streptomycin, and against highly purified streptomycin sulfate prepared from a crystalline salt.

Sensitivity of a Single Strain of E. Coli. The sensitivity of a given strain of *E. coli*§ was determined, by the method previously described, against three preparations of streptomycin on a number of days. As indicated in Table I, the sensitivity varied from 6.0 to 7.0 μg per cc against preparation I, from 4.0 to 8.0 μg per cc against preparation II, and from 4.0 to 9.0 μg per cc against preparation III.

Sensitivity of a Single Strain of E. Coli to 6 Preparations of Streptomycin Sulfate. In subsequent experiments the sensitivity of a single strain of *E. coli* was tested against six different preparations of streptomycin sulfate varying in potency from 249 to 802 μg per mg.

The sensitivity after 24 hours' incubation at 37°C. varied from 4.0 to 7.9 μg per cc. The action of streptomycin was only bacteriostatic, the amount necessary for inhibition increasing on prolonged incubation.

The sensitivity did not vary greatly with different preparations of streptomycin sulfate. A preparation containing 802 μg per mg and prepared directly from a crystalline salt of streptomycin was no more effective against this strain of *E. coli* than were more crude preparations. The variation in sensitivity to different preparations was no greater than the variation in sensitivity to a single preparation on different days. (Table II).

§ We are indebted to Dr. Selman Waksman for the strain of *E. coli* used in these experiments.

TABLE II.
Bacteriostatic Action of Various Preparations of Streptomycin on a Standard Strain of *E. coli in Vitro.*

Streptomycin preparation	Potency μg/mg	Sensitivity in μg/cc* 24 hr	48 hr	72 hr
I	249	5.8	6.5	6.5
II	455	6.5	7.8	7.8
III	404	7.9	7.9	7.9
IV	452	4.0	5.0	5.0
V	418	5.8	6.1	6.2
VI	802	6.3	6.3	6.3

* Sensitivity = Least amount of streptomycin causing complete inhibition of growth.
Values given represent average of 2 to 9 determinations made on separate days.

Effect of Concentration of Organisms on Sensitivity to Streptomycin. In view of the fact that the sensitivity of the same strain cultivated in the same medium was not absolutely constant from day to day, experiments were carried out to determine the effect of the density of the culture on its sensitivity to streptomycin.

The density of both 6 hour and 16 hour cultures of 2 strains belonging to different species—a standard strain of *E. coli* and a freshly isolated strain of *K. pneumoniae*—were diluted to the equivalent of a $BaSO_4$ No. 1 standard (78% transmission Photovolt Lumetron No. 400). This concentration was equivalent to 200-400 million organisms per cc. Six hour cultures were also diluted to the equivalent of a $BaSO_4$ No. 4 standard (50% transmission) whereas the 16 hour cultures were diluted to correspond to the density of a $BaSO_4$ No. 6 standard (28% transmission). The sensitivity of each to streptomycin was determined using 10^{-1} to 10^{-8} dilutions.

More constant and more complete inhibition of growth was obtained using 6 hour cultures. The number of μg per cc necessary to cause complete inhibition of growth decreased with a decrease in the number of organisms per cc. Using dilutions of 10^{-5} or greater (equivalent to less than 15,000 organisms per cc of final test solution), differences in the number of organisms had little effect on the

TABLE III.
Effect of Concentration of Organisms on Sensitivity to Streptomycin *in Vitro*.

Strain	Culture age, hr	Density, $BaSO_4$	Sensitivity in μg/cc* Dilution of culture							
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
E. coli (W.)	16	No. 1	10	5	5	4	2	2	2	2
		No. 6	10	10	5	4	2	2	2	2
	6	No. 1	10	10	5	2	2	2	2	2
		No. 4	10	10	10	5	2	2	2	2
K. pneum. (Lund.)	16	No. 1	10	10	5	2	2	2	2	1
		No. 6	10	10	5	4	3	2	2	2
	6	No. 1	10	10	5	3	2	2	2	2
		No. 4	10	10	10	4	2	2	2	2

* Sensitivity expressed in terms of least amount of streptomycin (μg/cc) necessary to cause inhibition of growth.

amount of streptomycin necessary for inhibition. In lower dilutions, a 10-fold difference in concentration of organisms was often sufficient to cause a 2-fold difference in the sensitivity of the organism when tested in the same medium under the same experimental conditions. (Table III).

The concentration of organisms, and to a less extent the age of the culture, is critical. Indeed it is more critical than in the case of penicillin—a 10,000-fold change in the concentration of an organism generally being essential to cause a 2- to 4-fold difference in its sensitivity to penicillin.

Variation in Sensitivity of Organisms Within Individual Strains. Throughout these studies, occasional irregularities resulted from the appearance of resistant cells at irregular intervals in the test series. In view of the fact that there is no evidence as yet that these cells differ in their pathogenicity, they cannot be ignored. However, the difference in sensitivity due to the appearance of such cells is no greater, in most cases, than the difference in sensitivity of a single strain when tested on different days or when tested against different preparations of streptomycin. (Table IV).

Effect of Peptone on Sensitivity of E. Coli to Streptomycin. A comparison of the sensitivity of the standard strain of *E. coli* was made in (1) a freshly prepared beef infusion broth, (2) Bacto-nutrient broth containing yeast extract, and (3) McLeod's synthetic medium for growth of *E. coli*. The sensitivity of this strain was greatest in the Bacto-

TABLE IV.
Influence of Resistant Cells on Sensitivity of Whole Culture to Streptomycin *in Vitro*.

Strain	Sensitivity in μg/cc (72 hr)	
	Susceptible cells*	Resistant cells†
E. coli (W.)	4.5	7
" " (Hopk.)	5.0	9
" *typhosa* (McM.)	7.0	9
" " (Ur.)	3.5	9
K. pneumoniae (Yer.)	4.5	7
Strep. viridans (Tor.)	2.5	10
Staph. aureus (K.C.)	5.0	10
" " (K.S.C.)	5.0	8
" " (Hand.)	10.0	200

* Least amount of streptomycin necessary to inhibit growth of apparently sensitive cells.
† Least amount of streptomycin necessary to inhibit growth of entire culture.

nutrient broth which contained only a low concentration of beef extract, peptone, and yeast extract at a pH of 6.9. In all 3 mediums, however, growth was inhibited by 5 μg of streptomycin per cc.

Using freshly prepared beef infusion broth, McLeod's synthetic broth, and a modified McLeod's medium in which the asparagin was replaced by neopeptone in amounts comparable to those present in the infusion broth, the sensitivity of varying dilutions of the standard strain of *E. coli* to streptomycin was tested.

The concentration of organisms ranged from a 10^{-1} to 10^{-8} dilution of a standard culture. Growth was inhibited in dilutions of 10^{-2} to 10^{-8} in beef infusion broth, and in dilutions from 10^{-3} to 10^{-8} in McLeod's medium containing asparagin. No inhibition

TABLE V.

Effect of Neopeptone on Action of Streptomycin (*E. coli*).

Basal medium	Amt of peptone (%)	Amt of streptomycin (μg/cc)	Amt of growth							
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
McLeod's	0	0	+++	+++	+++	+	+	+	+	+
"	0	5	+++	+++	+++	+	+	+	+	+
" *	0.2	0	+++	+++	+++	+	+	+	+	+
" *	0.2	5	+++	+++	+++	+	+	+	+	+
Beef inf.	1.0	0	+++	+++	+++	+	+	+	+	+
" "	1.0	5	++	++	++	+	+	+	+	+
	0	0	+++	+++	+++	+	+	+	+	+
	0	5	+++	+++	—	—	—	—	—	—
	0.2	0	+++	+++	+++	+	+	+	+	+
	0.2	5	+++	+++	+++	—	—	—	—	—
	1.0	0	+++	+++	+++	+	+	+	+	+
	1.0	5	++	++	++	—	—	—	—	—

* McLeod's synthetic medium containing no asparagin. Neopeptone used in place of asparagin.

of growth occurred in McLeod's medium containing 0.2% neopeptone in place of asparagin. (Table V). Similar results were obtained using 1.0% neopeptone in McLeod's medium.

The inhibition of streptomycin action by peptone was in striking contrast to the effect obtained in beef infusion broth containing an equal concentration of the same preparation of peptone and containing the same concentration of glucose. That peptone may alter the oxidation-reduction potential of a medium was shown by Dubos.[14] It has been demonstrated by Geiger, Green and Waksman[15] and by others[9,12,16] that such alteration in the oxidation-reduction potential of the medium may inhibit the action of streptomycin. It is conceivable that the inhibitory effect of peptone on the antibacterial action of streptomycin may be due at least in part to such an alteration in the oxidation-reduction potential of the medium. Beef infusion broth apparently contains however one or more substances which will counteract the effect of the peptone on the action of streptomycin.

An inhibitory effect similar to that observed with peptone was also obtained when asparagin was replaced by methionine, cystine HCl, and tyrosine. No inhibition of streptomycin action occurred with the concentrations of leucine, glutamic acid, tryptophane, glucose, or paraminobenzoic acid tested. Certain other of the amino acids were tested but were in themselves inadequate to support growth of the organism in the synthetic medium used.

It is apparent that the medium used in testing the sensitivity of an organism to streptomycin is extremely important. The fact that certain growth stimulating substances such as peptone may have an inhibitory effect on streptomycin seems highly significant. Since large differences in sensitiv-

[13] Dawson, M. H., *J. Path. and Bact.*, 1935, **39**, 323.

[14] Dubos, R., *J. Exp. Med.*, 1930, **52**, 331.

[15] Geiger, W. B., Green, S. R., and Waksman, S. A., *J. Bact.*, 1946, **51**, 634.

[16] Denkelwater, R., Cook, M. A., and Tischler, M., *Science*, 1946, **102**, 12.

ity levels may result from the use of different mediums in different laboratories, it is essential that the medium is indicated or that a reference standard is used. In a constant medium the variations in sensitivity of a given organism are not significantly great from day to day provided age and density of culture are controlled.

Sensitivity of Freshly Isolated Strains. In subsequent experiments the sensitivity of 84 freshly isolated strains was tested in beef infusion broth using a 10^{-3} dilution of a 6 hour culture diluted to 78% transmission as previously described. The sensitivity was accepted as the least amount of streptomycin necessary to cause inhibition of growth as evidenced by absence of turbidity after 72 hours at 37°C. The sensitivity was not expressed in terms of the actual number of μg per cc necessary for inhibition of growth, but in terms of the ratio of the number of micrograms necessary to inhibit the unknown organism being tested to the number necessary to inhibit the standard strain of *E. coli* in the same medium on the same day. Expressing sensitivity in terms of such a ratio does not eliminate differences in the culture from day to day or differences due to the appearance of occasional resistant cells, but it will eliminate differences caused by medium. The sensitivity of the standard strain of *E. coli* under such conditions is then 1.0 at all times.

TABLE VI.
Comparison of Bacteriostatic Action of Streptomycin on Various Species of Bacteria *in Vitro.*

Species	No. of strains tested	Avg sensitivity* 24 hr	72 hr
H. influenzae	1	0.2	0.2
B. mycoides	1	0.4	0.4
K. pneumoniae	5	0.3	0.5
Aer. aerogenes	1	0.6	0.6
E. typhosa	7	2.6	5.5
E. coli	10	1.6	2.2
Streptococcus	4	2.3	3.4
Salmonella	5	2.3	3.4
Staph. aureus	44	1.0	3.5
Pseud. pyocyaneus	6	13.9	24.1

* Sensitivity = avg of ratios of least amount of streptomycin (μg per cc) causing complete inhibition of growth of unknown strain to least amount (μg per cc) causing inhibition of a standard *E. coli* strain on the same day.

TABLE VII.
Comparison of Sensitivity of Various Strains Within a Species to Streptomycin *in Vitro.*

Species	Strain No.	Sensitivity (72 hr)*
E. coli	1	0.2
	2	0.6
	3	0.7
	4	0.7
	5	0.8
	6	1.0
	7	1.0
	8	5.6
	9	7.5
Ps. pyocyaneus	1	5.6
	2	7.5
	3	100.0

* Sensitivity = ratio of least amount of streptomycin (μg per cc) causing complete inhibition of growth of unknown strain to least amount (μg per cc) causing inhibition of standard *E. coli* strain on same day.

TABLE VIII.
Difference in Bacteriostatic Action of Streptomycin on Individual Organisms Within a Strain of *Staph. aureus.*

Strain	No. of colonies tested	Sensitivity* 24 hr	48 hr	72 hr
K.L.	2	0.3	0.3	0.5
		0.2	4.3	18.8
K.N.	2	0.6	0.6	0.8
		4.2	5.0	22.5

* Sensitivity = ratio of least amount of streptomycin (μg per cc) causing complete inhibition of growth of unknown strain to least amount (μg per cc) causing inhibition of standard *E. coli* strain on same day.

The average sensitivity of 10 strains of *E. coli,* other than the standard strain, was 2.2 μg per cc. These strains were 2.2 times as resistant as the standard strain. The strains of *H. influenzae, B. mycoides, K. pneumoniae,* and *Aer. aerogenes* tested were more sensitive whereas the streptococci (all of which were enterococci with the exception of one strain), staphylococci, Salmonella, and Pseudomonas strains tested were more resistant than the standard strain of *E. coli.* (Table VI).

In some instances marked variations occurred in the sensitivity of the various strains within a single species. (Table VII). Likewise, there were at times marked differences in the sensitivity of individual organisms within a single strain. (Table VIII).

Comparison of Sensitivity to Crude and

TABLE IX.
Comparison of Bacteriostatic Action of Crude and Purified Streptomycin *in Vitro*.

Strain	Sensitivity in μg per cc*				
			Crystalline ($CaCl_2$ double salt)		
	Crude sulfate 453 μg/mg	Pure sulfate 802 μg/mg	Prep. 1 685 μg/mg	Prep. 2 701 μg/mg	Prep. 3 708 μg/mg
B. subtilis (W)	0.14	0.25	0.25	0.19	0.11
K. pneumoniae	0.17	0.16			
B. mycoides (W)	0.21	0.25	0.25	0.25	0.17
A. aerogenes	0.33	0.29	0.25	0.19	0.22
" "	0.52	0.32			
E. coli (W)	1.00	1.00	1.00	1.00	1.00
Staph. aur. (H)	2.20	2.50	2.50	2.50	2.00
Strep. hem. (C203Mv)	2.40	2.10			
E. typhosa	5.70	4.00	>5.00	5.00	>5.00

* Sensitivity = ratio of least amount of streptomycin (μg per cc) causing complete inhibition of growth of unknown strain to least amount (μg per cc) causing inhibition of standard strain of *E. coli* on same day.

Pure Streptomycin. The sensitivity of a small group of organisms against a partially purified preparation of streptomycin sulfate (potency, 453 μg per mg) was compared with their sensitivity to 3 preparations of the crystalline $CaCl_2$ double salt of streptomycin and to one preparation of pure streptomycin sulfate prepared from a crystalline salt. The difference in sensitivity was insignificant and it is apparent that the strains tested were equally sensitive to the preparations of impure and pure streptomycin used. (Table IX).

Subsequent experiments using different preparations of impure streptomycin revealed a somewhat different situation however. Using 5 different strains of *E. typhosa* as test organisms, the sensitivity to 2 preparations of impure streptomycin sulfate and to one preparation of highly purified streptomycin sulfate was compared. The sensitivity of all 6 strains to the preparation of highly purified streptomycin sulfate was >36 μg per cc. Likewise the sensitivity to one preparation of impure streptomycin sulfate ranged from 24 to 36 μg per cc. However the sensitivity to the other preparation of impure streptomycin sulfate ranged from 3.3 to 7.0 μg per cc. It was apparent that some preparations of impure streptomycin sulfate were more effective than others against *E. typhosa.* (Table X).

TABLE X.
Comparison of Bacteriostatic Action of Crude and Purified Streptomycin Against *E. Typhosa in Vitro.*

Strain	Sensitivity in μg/cc (72 hr)*		
		Impure sulfate	
	Pure sulfate 802 μg/mg	Prep. 1 130 μg/mg	Prep. 2 453 μg/mg
E. typhosa (stock)	>40		32
(Ga)	>40	4.3	36
(Me)	>36	3.3	24
(Mc)	>40	7.0	24
(Ur)	>36	3.8	40

* Sensitivity expressed in terms of least amount of streptomycin (μg per cc) necessary to cause inhibition of growth.

Discussion. The sensitivity of an organism to streptomycin is influenced by many factors including (1) age of culture, (2) concentration of organisms, (3) growth phase of the culture, and (4) constituents of the medium used. Provided the age, concentration and growth phase of the organisms are constant, sensitivities will remain constant from day to day if the same medium is used throughout. Since differences in medium alter so greatly the sensitivity of an organism, comparison of sensitivities is facilitated by expressing them in relation to a standard strain tested simultaneously in the same medium.

The effect of streptomycin is highly bacteriostatic. Only against a few organisms (*H. influenzae, Pasteurella tularensis, A. aerogenes*) has bactericidal activity been demonstrated.[8] The fact that with many strains the number of organisms in relation to the

concentration of drug so greatly influences the effect of the streptomycin is probably a limiting factor in its usefulness. In like manner the effectiveness of the sulfonamides has been dependent at least in part on the concentration of organisms in relation to the amount of sulfonamide present. The number of units of penicillin necessary for inhibition of a culture, on the other hand, is altered only by large variations in the number of organisms present.

One can only speculate on the relationship this may have to the apparent development of bacterial resistance to streptomycin. It is now recognized however that in contrast to penicillin, the effectiveness of streptomycin *in vivo* is at times limited by the ease with which susceptible strains of microorganisms develop resistance to it.

Under the experimental conditions used, no differences have been demonstrated in the sensitivity of most organisms to impure streptomycin sulfate, to the crystalline $CaCl_2$ double salt of streptomycin, or to highly purified streptomycin sulfate prepared from a crystalline salt. Impure streptomycin and highly purified or crystalline streptomycin appear equally effective *in vitro* against all organisms except *E. typhosa*. The significance of the fact that certain preparations of impure streptomycin sulfate are more effective against strains of *E. typhosa* than highly purified streptomycin will be discussed in detail elsewhere.

Summary. 1. A standardized procedure for determination of sensitivity of microorganisms to streptomycin is described.

2. The sensitivity of an organism to streptomycin is influenced by age of culture, concentration of organisms, growth phase of culture, and constituents of medium used. Providing these factors are held constant, the sensitivity of a given strain will remain constant from day to day.

3. The action of streptomycin is bacteriostatic rather than bactericidal. Its action is inhibited by certain growth stimulating substances such as peptone, as well as by certain reducing substances.

4. The sensitivity of 84 strains belonging to 7 species is described. Marked variation in sensitivity exists between different strains within a single species and at times between different cells within a given strain.

5. The sensitivity of 9 strains belonging to 8 species is essentially the same when tested against crude streptomycin sulfate (453 μg/mg), against 3 preparations of the crystalline $CaCl_2$ double salt of streptomycin (685 to 708 μg/mg) and against a preparation of streptomycin sulfate (802 μg/mg) prepared from a crystalline salt.

6. The sensitivity of 4 strains of *E. typhosa* to certain preparations of impure streptomycin sulfate is greater than to highly purified streptomycin sulfate.

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Observations on the Action of Streptomycin *in vitro* (II).*

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It is recognized that a wide variety of factors may influence the observed sensitivity of an organism to streptomycin. Certain of these factors have been discussed in a recent communication from this laboratory.[1] The sensitivity of a number of strains belonging to 10 different species was described and it was shown that with the exception of *E. typhosa* the strains tested were equally sen-

* A part of this work was presented at the Conference on Antibiotic Research held at Washington, D.C. on January 31 and February 1, 1947 under the auspices of the Antibiotics Study Section of the National Institute of Health.

[1] Lenert, T. F., and Hobby, G. L., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 235.