Piromen has been shown here to increase the phagocytic activity of both fixed macrophages and leukocytes. While no beneficial effect was noted in mice infected with *K. pneumoniae* the ability of this drug to increase phagocytic activity *in vivo* suggests that it should be evaluated under a variety of experimental conditions.

Summary. Cortisone, ascorbic acid and Piromen were tested in mice for their effect upon macrophage activity as determined from the splenic uptake of colloidal ThO_2 and upon the phagocytic activity of leukocytes in the peritoneal cavity against *Micrococcus aureus*. Cortisone in high doses (0.1 to 1 mg every 12 hours) significantly enhanced phagocytic activity of both macrophages and leukocytes. Ascorbic acid in doses as high as 1 mg every 12 hours had no effect on the activity of macrophages but this dose did significantly enhance the phagocytic ability of leukocytes in the peritoneal cavity. Piromen (0.1 μg every 12 hours) significantly increased the phagocytic activity of both types of cells. However, doses above or below this value were without significant effect in either type of experiment. The more potent auxophagocytic agents (cortisone and Piromen) were without salutary effect when tested singly and in combination in protection experiments in mice challenged with predetermined doses of *Klebsiella pneumoniae*.

1. Gordon, A. S., and Katsh, A. F., *Ann. New York Acad. Sciences*, 1949, v52, 1.
2. Esplin, D. W., and Marcus, S., *J. Immunol.*, 1953, v70, 454.
3. Berry, L. J., Starr, R. W., and Haller, E. C., *J. Bact.*, 1949, v57, 603.
4. Hoogerheide, J. S., *ibid.*, 1939, v38, 367.
5. ———, *ibid.*, v39, 649.
6. Fisher, R. A., *Statistical methods for research workers*, 11th ed., 1950, Hafner, New York.
7. Metchnikoff, E., *Immunity in Infective Diseases*, 1905, Cambridge University Press, England.
8. Nungester, W. J., and Ames, A. M., *J. Infect. Dis.*, 1952, v90, 51.
9. ———, *ibid.*, 1948, v83, 50.
10. Merchant, D. J., *ibid.*, 1950, v87, 275.

Received October 19, 1953. P.S.E.B.M., 1953, v84.

Differentiation of Streptomycin and Dihydrostreptomycin Inhibition, by Means of Reversing Metabolites.* (20713)

R. F. PITTILLO AND J. W. FOSTER. (Introduced by W. W. Umbreit.)
From the Department of Bacteriology, University of Texas, Austin.

The close chemical relation between streptomycin (SM) and dihydrostreptomycin (DSM) together with the close resemblance of their antimicrobial spectra with respect both to wild type organisms and to strains developed for resistance to each respectively, has led to the widespread current belief that these antibiotics inhibit bacteria by identical mechanisms. The activity of DSM has even been postulated to result from its oxidation to SM by the bacteria(1).

SM and DSM have been extremely difficult to distinguish microbiologically, if indeed it has, with assurance, been possible at all, and frequently the two have been regarded as identical in antimicrobial growth effects(2-6) and in enzymatic effects(7), as well as in dependency experiments(8). Also SM and DSM appear to be absorbed and excreted by the mouse in the same way(6).

On the other hand, there is some evidence suggesting that SM and DSM may not be identical in their antibacterial action. This evidence rests principally on small differences in relative amounts of the two antibiotics required for inhibition of a number of different organisms tested in parallel(6,9). Of interest in this connection is the fact that the neurotoxic threshold dose (especially for the eighth nerve) of DSM in animals is distinctly less than for SM(10).

* This work supported in part by funds from Atomic Energy Commission; Office of Naval Research; American Cancer Society recommended by the Committee on Growth of the National Research Council.

TABLE I. Selective Reversal, by Individual Metabolites, of *Aerobacter aerogenes* Growth Inhibition Caused by Streptomycin and by Dihydrostreptomycin. Plates incubated for 34 hr at 37°C.

Reversing agent, μg/ml agar medium	Antibiotic and μg/ml agar medium											
	No antibiotic	Streptomycin						Dihydrostreptomycin					
		.5	1	2	4	8	16	.5	1	2	4	8	16
None	+	+	—	—	—	—	—	+	—	—	—	—	—
Guanylic acid, 5	+	+	+	+	+	+	—	+	—	—	—	—	—
Cytosine, 5	+	+	+	+	+	+	—	+	—	—	—	—	—
Xanthosine, 5	+	+	+	+	+	+	—	+	—	—	—	—	—
Guanosine, 5	+	+	+	+	+	+	—	+	—	—	—	—	—
Thymine, 5	+	+	+	+	+	—	—	+	—	—	—	—	—
L-phenylalanine, 25	+	+	—	—	—	—	—	+	+	+	+	—	—
L-phenylalanine, 50	+	+	—	—	—	—	—	+	+	+	+	+	—
Yeast autolysate, 1000	+	+	+	+	+	+	—	+	+	—	—	—	—

+ = Growth of approximately 100 discrete colonies.
— = No colonies visible to the naked eye.

This paper reports a qualitative distinction between SM and DSM with respect to reversibility, by selected known metabolites, of the growth inhibition caused by these antibiotics. As used here, the term "reversal" simply refers to the prevention or counteraction of the growth inhibition (irrespective of mechanism) otherwise caused by a given concentration of an antibiotic.

Materials and methods. The spread plate procedure, employing *Aerobacter aerogenes* strain P and M-9 agar was used for screening various known metabolites for their ability to reverse or neutralize otherwise inhibitory concentrations of the particular antibiotics. Composition of M-9 agar, grams per liter of distilled water: dextrose 4.0, NH_4Cl 1.0, $MgSO_4 \cdot 7\ H_2O$ 0.12, Na_2HPO_4 6.0, KH_2PO_4 3.0, agar 15.0. Antibiotics and reversing agents were added as aqueous solutions (0.5 to 1.0 ml) to sterile petri dishes and 10 ml cooled melted agar added. The supplements were mixed thoroughly with the agar by rotating the plates. The agar was then allowed to harden. The plates were spread with 0.05 ml of an aqueous suspension containing approximately 100 washed cells. The procedures were exactly like those described previously(11,12). The term "reversal factor" connotes the ratio of amount of antibiotic required to inhibit in the presence of a reversing agent to that required in the absence of the agent. Colonies in plates containing antibiotic and reversing agent become visible about 4 to 6 hours later than appearance of visible colonies in the basal medium. After the delay, rate of growth of the "reversed" colonies approximates that of the controls, and in about 24 to 30 hours colonies in both are about the same size.

Results. Differential reversal of growth inhibition caused by SM and by DSM. Of fifty metabolites tested in preliminary screening experiments(11,12) only L-phenylalanine and a group of purine and pyrimidine derivatives were found to possess reversing activity against SM and DSM inhibition of *A. aerogenes*. Table I presents the particular reversing compounds, the degree of reversal, and their specificity with respect to SM and DSM. For example, in the absence of reversing agents, 1.0 μg of SM and DSM respectively sufficed for complete inhibition. However, in the presence of the purine and pyrimidine derivatives 16 μg SM was required for inhibition. The presence of these derivatives did not, however, alter the amount of DSM required for inhibition, namely 1 μg, the same as in the absence of the derivatives. Similarly, phenylalanine did not influence the inhibitory level of SM, it being 1 μg irrespective of the presence or absence of this amino acid. However, phenylalanine did increase markedly the amount of DSM required for inhibition as compared to in its absence, *e.g.*, 16 μg vs. 1 μg.

It is evident that guanylic acid, cytosine, xanthosine, guanosine and thymine each displayed reversal of SM inhibition, with reversal factors of 16 for all except thymine and that these compounds displayed no detactable reversal of DSM tested simultaneously. Conversely, L-phenylalanine reversed only DSM inhibition (reversal factor = 16) and had no

activity in reversing SM inhibition. The reversing activity of the amino acid was proportional to the concentration used, within the limited range tested. A concentration of 100 μg per ml agar was toxic in the growth control plates. Also, a commercial yeast autoylsate (Basamine-Busch) was 8-fold more effective in reversing SM than DSM. In other experiments, varying concentrations of the reversing agents did not alter the qualitative specificity of the results.

Discussion. The evidence here, together with the absence of cross-reversal with SM- and DSM-reversing substances produced by homologous resistant strains described elsewhere(13), while not conclusively establishing different mechanisms of action of SM and DSM, does contradict the widespread assumption that both act in identical fashion. The reversing compounds may well be related to the biochemical mechanisms interrupted when the cells are inhibited by threshold concentrations of the antibiotics.

Summary. Several purine and pyrimidine derivatives reversed the growth inhibition of *Aerobacter aerogenes* caused by streptomycin under specified conditions. Inhibition by dihydrostreptomycin was not reversed under the same conditions. L-phenylalanine reversed the inhibition caused by dihydrostreptomycin and not that caused by streptomycin.

1. Henry, R. J., and Hobby, G. L., in *Streptomycin*, p215, Ed. by Waksman, Williams and Wilkins, Baltimore, 1949.

2. Szybalski, W., and Bryson, V., *J. Bact.*, 1952, v64, 489.

3. Bartz, Q. R., Controulis, J., Crooks, H. M., and Rebstock, M. C., *J. Am. Chem. Soc.*, 1946, v68, 2163.

4. Edison, A. D., Frost, B. M., Grassle, O. E., Hawkins, J. E., Kuna, S., Mushett, C. W., Silver, R. H., and Solotorovsky, M., *Am. Rev. Tuberc.*, 1948, v58, 487.

5. Feldman, W. H., Karlson, A. G., and Hinshaw, H. C., *ibid.*, 1948, v58, 494.

6. Rake, G., Pansy, F. E., Jambor, W. P., and Donovick, R., *ibid.*, 1948, v58, 479.

7. Umbreit, W. W., *Ann. N. Y. Acad. Sci.*, 1950, v53, 6.

8. Miller, C. P., and Bohnhoff, M., in *Streptomycin*, 1949, p158-176, Loc. Cit.

9. Miles, A. A., in *Microbial Growth and its Inhibition*, 1952, p135, World Health Organization Monograph Series No. 10, Geneva.

10. Molitor, H., in *Streptomycin* 1949, p272-3, Loc. Cit.

11. Foster, J. W., and Pittillo, R. F., *J. Bact.*, 1953, v65, 361; v66, in press.

12. Pittillo, R. F., and Foster, J. W., *ibid.*, 1953, v66, in press.

13. Foster, J. W., and Pittillo, R. F., unpublished experiments.

Received October 26, 1953. P.S.E.B.M., 1953, v84.

Isolation of a Cytopathogenic Agent from Human Adenoids Undergoing Spontaneous Degeneration in Tissue Culture. (20714)

WALLACE P. ROWE, ROBERT J. HUEBNER, LORETTA K. GILMORE, ROBERT H. PARROTT, AND THOMAS G. WARD. (Introduced by Elizabeth Verder.)

From the Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Bethesda, and the Department of Microbiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.*

During the course of a study of the growth of human adenoid tissue in roller tube culture, a characteristic degeneration has been encountered which has been found to be serially transmissible in other tissue cultures.

Methods. Adenoids were obtained during the winter and spring of 1952-53 from operations on young children.† The tissues were

* Laboratory of Infectious Diseases and the Laboratory of Clinical Investigations.

† We are glad to acknowledge the cooperation of Dr. E. Clarence Rice, pathologist, Children's Hospital, Washington, D. C., the Research Foundation, Children's Hospital, Washington, D. C., and the U. S. Naval Hospital, Bethesda, Md., in supplying the adenoids.