quired, by placing the respirometer on any commercial shaker or vibrator with adequate speed of motion.

Example of use. The adapted instrument was used to measure oxygen uptake of human gingiva obtained by surgical excision(8). Position of micrometer plunger at various time intervals and cumulative oxygen uptake at 38°C are shown in Table I. Wet weight of the respiring sample was 7.28 mg or 0.223 mg nitrogen as determined by Kjeldahl nesslerization.

Summary. A Stern-Kirk microrespirometer was provided with a plunger adaptation which obviates the need of prior calibration of volumes of respiration and compensation chambers. By combining interchangeable plungers of different diameters with various available capillary manometer tubes, the range of the instrument can be extended. Use of the instrument in measuring the oxygen uptake of human gingival tissue is described.

1. Stern, H., Kirk, P. J., *Gen. Physiol.*, 1948, v31, 239.
2. Winterstein, H., *Biochem. Z.*, 1912, v46, 440.
3. Scholander, P. F., *Rev. Sci. Instr.*, 1942, v13, 32.
4. ———, *ibid.*, 1949, v20, 885.
5. Wennesland, R., in *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*, Umbreit, W. W., Burris, R. H., Stauffer, J. F., Eds., Minneapolis, Burgess Pub., 1948.
6. ———, *Science*, 1951, v114, 100.
7. Gilmont, R., *Anal. Chem.*, 1948, v20, 1109.
8. Senter, A. D., Eiler, J. J., Lee, K.-H., PROC. SOC. EXP. BIOL. AND MED., in press.

Received December 8, 1958. P.S.E.B.M., 1959, v100.

Reversal of Inhibitory Activity of Chlorpromazine by Calcium.* (24679)

H. VASKEN APOSHIAN, NANCY S. POINTER AND MARY M. APOSHIAN
(Introduced by A. D. Bass)
Dept. of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tenn.

Since the use of microorganisms has been a valuable aid in discovery of new and the elucidation of known metabolic reactions, a program has been initiated in which bacteria were used to obtain information as to possible mode of action of various drugs affecting the central nervous system. The present report concerns inhibition of growth of *E. coli* by chlorpromazine, a "tranquilizer," and its reversal by calcium.

Methods. *E. coli* A.T.C.C. #9723 was maintained and the inoculum prepared essentially by a previously described method(1). All assays were performed at 37° for 17 hours in final volume of 10 ml, using Dunn's(1) modification of Anderson's salts, ammonium chloride and glucose medium(2).

Results. As shown in Fig. 1, 0.075 μmole of chlorpromazine/ml of medium produces 50% inhibition of maximum growth of this organism, whereas 0.14 μmole of promazine, a less active "tranquilizer" for humans, is necessary for 50% inhibition of growth. Since these 2 drugs also possess antihistaminic activity, tripelennamine (a commonly used antihistaminic) was tested and found devoid of significant inhibitory activity under our conditions.

Various compounds, either metabolites similar in structure, neurohumoral agents or substituents of coenzymes, when used in concentrations that alone were not inhibitory for this strain of *E. coli*, did not reverse inhibitory activity of chlorpromazine or promazine. Examples of such compounds are histamine, histidine, acetylcholine, epinephrine, norepinephrine, 5-hydroxy-tryptamine, nicotinic acid, nicotinamide, thiamine, riboflavin, biotin, pyridoxine, pyridoxal phosphate, or pyridoxamine.

Calcium-d-pantothenate, however, reversed the inhibitory activity of chlorpromazine. Since the amount of pantothenate required for reversal was greater than that expected to be

* This study was supported in part by Nat. Cancer Inst.

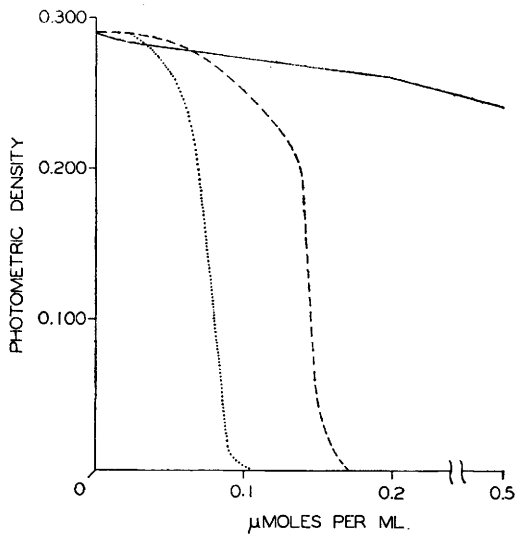

FIG. 1. Influence of chlorpromazine, promazine or tripelennamine on 17-hr growth of *E. coli* #9723. Chlorpromazine (•••••), promazine (– – – –), tripelennamine (———).

TABLE I. Reversal of Inhibitory Activity of Chlorpromazine by Calcium-d-pantothenate, Calcium Chloride or Calcium Acetate.

Chlorpromazine	Amt of supplement	Supplements: Calcium-d-pantothenate	Supplements: Ca. chloride	Supplements: Ca. acetate
μmoles/ml		Photometric density*		
.0	.0	.299	.299	.299
.1	.0	.008	.008	.008
.1	.1	.030	.040	.038
.1	.2	.174	.156	.192
.1	.3	.200	.204	.215
.1	.4	.234	.238	.244
.1	.5	.265	.270	.267
.1	.6	.281	.273	.272
.0	.1	.310	.301	.301
.0	.2	.299	.303	.310
.0	.3	.308	.304	.304
.0	.4	.309	.305	.308
.0	.5	.306	.310	.311
.0	.6	.306	.303	.303

Test organism, *E. coli* #9723, incubated for 17 hr at 37°.

* A measure of culture turbidity. Photometric density = 2 − log G where G equals galvanometer reading of Evelyn Photoelectric Colorimeter.

necessary for catalytic purposes, it was thought that reversal of the inhibitory activity of chlorpromazine might be due to the calcium ion rather than the pantothenate moiety. The reversing activity of calcium-d-pantothenate was compared with that of calcium chloride or calcium acetate. Table I shows that calcium in the form of calcium-d-pantothenate, calcium chloride, or calcium acetate reverses the inhibitory activity of chlorpromazine. Sodium chloride or sodium acetate is unable to reverse inhibitory activity of chlorpromazine.

It is of interest to note that chlorpromazine is twice as active as promazine as an inhibitor of this organism and that the former is twice as potent as the latter as an ataractic in humans. Woolley has recently shown that the muscle contracting activity of 5-hydroxytryptamine is associated with a change in permeability of the muscle cell to calcium ions (3). It would be of interest to investigate the relationship between chlorpromazine and permeability of mammalian cell membranes to calcium to elucidate the mode of action of this "tranquilizer" in humans.

Summary. Chlorpromazine inhibits growth of *E. coli* 9723. Various compounds, either metabolites similar in structure, neurohumoral agents or substituents of coenzymes, do not reverse inhibitory activity of this "tranquilizer." Calcium in the form of calcium-d-pantothenate, calcium chloride or calcium acetate reverses the inhibitory activity of chlorpromazine.

1. Dunn, F. W., Ravel, J. M., Shive, W., *J. Biol. Chem.,* 1956, v219, 809.

2. Anderson, E. H., *Proc. Nat. Acad. Sci.,* 1946, v32, 120.

3. Woolley, D. W., *Science,* 1957, v126, 1236.

Received December 11, 1958. P.S.E.B.M., 1959, v100.