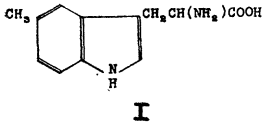
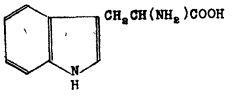
THE ACTIVITY OF A BACTERIOSTATIC SUBSTANCE IN THE REACTION BETWEEN BACTERIAL VIRUS AND HOST[1]

MOST bacteriostatic agents are supposed to act in suppressing growth by interfering with the metabolism of an essential substance either by combining with it chemically,[2] or by competing with it for positions on enzyme surfaces by virtue of the structural similarity of the agent to that of the essential metabolite (see reference 3 for literature references).

The results of the present work appear to bear on this point. It is found that while Bz-3-methyltryptophane (I) suppresses the growth of *B. coli* strain B in the absence of added tryptophane and does not do so in the presence of traces of tryptophane (II), it



I



II

appears to act as tryptophane does[4] in promoting the action of the bacterial viruses T4 and T6 on this strain of *E. coli*.

The Bacteriostatic Action of Bz-3-methyltryptophane and Its Reversal by Tryptophane. Preliminary experiments showed that Bz-3-methyltryptophane inhibited the growth of *E. coli* on solid or liquid ammonium lactate medium,[5] but that it did not do so on Difco nutrient agar. Such inhibition was observable at concentrations of dl-Bz-3-methyltryptophane as low as 4 micromoles/liter and, as seen in Table 1, was reversed at this and higher concentrations by *l*-tryptophane at concentrations about 1/1000th those of the *dl*-Bz-3-methyltryptophane. It thus appears probable that the methyltryptophane interferes in some manner with either the synthesis or utilization of tryptophane by *E. coli* grown on ammonium lactate medium.

The Effect of Bz-3-methyltryptophane on the Activity of Bacterial Viruses. The strain B of *E. coli* used in the above experiments is susceptible to the action of three bacteriophages, T2, T4 and T6[6] which belong to the same serological group and have similar morphologies.[7] T2 is rapidly absorbed on B in the ammonium lactate medium, while T4 and T6 are very slowly adsorbed in the medium unless *l*-tryptophane at 50 micromoles/liter or some other co-factor (phenylalanine is weakly active) is present in the adsorption mixture.[4] This reaction seemed to be quite specific, for none of a long list of other amino acids, growth substances or known precursors of tryptophane proved to be capable of acting as co-factors for T4.

Turning to the study of a series of substances chemically related to tryptophane, Bz-3-methyltryptophane appeared to be highly active in promoting the adsorption of T4 on B. In experiments patterned after those described elsewhere[4] it was found that in 5 minutes Bz-3-methyltryptophane at a concentration of 200 micromoles/liter brought about the adsorption of about 50 per cent. of the particles in a mixture of T4 and B in the ammonium lactate medium, while the adsorption in controls without a co-factor was negligible. Moreover, virus particles so adsorbed went on to the production of plaques on a bacterial smear just as though the co-factor had been *l*-tryptophane.

A convenient method for comparing the activities of various co-factors is provided by the lytic reaction of

TABLE 1

GROWTH OF *E. coli* IN AMMONIUM LACTATE MEDIUM CONTAINING MIXTURES OF *l*-TRYPTOPHANE AND *dl*-Bz-3-METHYLTRYPTOPHANE

l-tryptophane (micromoles/liter)	Bz-3-methyltryptophane (micromoles/liter)					Bz-3-methyltryptophane (micromoles/liter)					Bz-3-methyltryptophane (micromoles/liter)				
	460	92	18	3.7	0	460	92	18	3.7	0	460	90	18	3.7	0
	Growth after 22 hours					Growth after 43 hours					Growth after 3 days				
2	2	2	2	2	2	4	4	4	4	5	4	5	4	4	6
0.4	2	2	2	2	2	2	4	3	3	5	4	5	4	4	6
0.08	0	1	1	1	2	0	1	3	3	5	1	4	4	4	6
0.016	0	1	1	1	2	0	1	3	3	4	1	3	4	4	4
0.0032	0	0	0	0	2	1	0	0	1	4	1	2	1	4	5
0	0	0	0	0	2	0	0	0	2	4	2	0	0	3	4

Initial inoculum 10^6 bacteria to 1 cc ammonium lactate medium in each tube.
0 = no visible growth, 1 = trace, . . . , 6 = heavy growth.

[1] From the Eldridge Reeves Johnson Research Foundation, University of Pennsylvania, Philadelphia.

[2] Fildes, *Brit. Jour. Exp. Path.*, 21: 67, 1940.

[3] D. W. Woolley, SCIENCE, 100: 579, 1944.

[4] T. F. Anderson, *Jour. Cell. and Comp. Physiol.*, 25: 17, 1945.

[5] T. F. Anderson, *ibid.*, 25: 1, 1945.

[6] M. Demerec and U. Fano, *Genetics*, 30: 119, 1945.

[7] T. F. Anderson, M. Delbrück and M. Demerec, "Types of Morphology Found in Bacterial Viruses." Paper given at the second annual meeting of the Electron Microscope Society of America, Chicago, on November 17, 1944.

certain virus suspensions on host cells which have been heavily irradiated with ultraviolet light. Not requiring a co-factor, T2 virus rapidly lyses *E. coli* B irradiated with some 200 to 500 lethal doses of ultraviolet light. Such lysis proceeded in a summary manner, *i.e.*, without the multiplication of virus which accompanies lysis of normal host cells. In the case of T2 virus, this reaction is accomplished by a lytic substance which is separable from the major portion of the virus particles.[5]

T4 and T6 virus display no such activity in the absence of a suitable co-factor to enhance their rates of adsorption on the host cells; with a co-factor present, T4 and T6 each react in a manner related to the concentration and activity of the co-factor. The results of a comparison of the activities of *dl*-tryptophane and *dl*-Bz-2-methyltryptophane are given in Fig. 1. It is

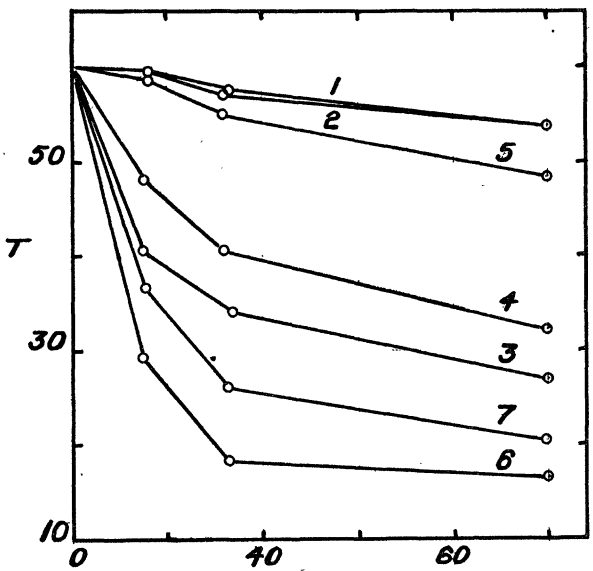


FIG. 1. The lysis of UV-irradiated *E. coli* strain B by viruses T4 and T6 in the presence of co-factors *dl*-tryptophane and *dl*-Bz-3-methyltryptophane. The bacteria in an actively multiplying state in ammonium lactate medium were irradiated in a quartz tube with about 500 lethal doses of ultraviolet light from a H-4 lamp with the outer glass shell removed. 5 cc of the irradiated bacterial suspension were then added to each of seven colorimeter tubes which contained in 0.4 cc of the ammonium lactate medium: 1. Control. 2. 0.95×10^{10} T4 virus particles. 3. 0.95×10^{10} T4 virus particles + 0.204 mg *dl*-tryptophane. 4. 0.95×10^{10} T4 virus particles + 0.201 mg *dl*-Bz-3-methyltryptophane. 5. 0.90×10^{10} T6 virus particles. 6. 0.90×10^{10} T6 virus particles + 0.204 mg *dl*-tryptophane. 7. 0.90×10^{10} T6 virus particles + 0.201 mg *dl*-Bz-3-methyltryptophane. The turbidities of tubes were read at intervals on a Klett-Summerson Colorimeter (blue filter No. 42) and in the figure are plotted as ordinates against minutes after mixing plotted as abscissae.

seen that the methyltryptophane at 170 micromoles/liter is only slightly less active than *dl*-tryptophane itself at 185 micromoles/liter. These experiments show that Bz-3-methyltryptophane can take the place of tryptophane in the adsorption reaction between the viruses T4 and T6 and their host.

Discussion. Gordon and Jackson[8] showed that Bz-3-methyltryptophane can not replace tryptophane in the diet of the rat. Indeed, the death of three out of four animals fed diets in which Bz-3-methyltryptophane replaced tryptopnane suggested to them that the compound might be somewhat toxic. However, in further work they found that the substance had no effect on the growth rate of rats fed an adequate diet. The above experiments on the reversal of the methyltryptophane inhibition of *E. coli* growth by traces of tryptophane suggest that the tryptophane in Gordon and Jackson's complete diet for rats may have masked the detrimental effects of Bz-3-methyltryptophane.

The fact that Bz-3-methyltryptophane has almost the activity of tryptophane as a co-factor for T4 and T6 adsorption on their hosts is interesting from a number of standpoints. It indicates that this inhibitor of bacterial growth actually does perform the function of the structurally similar essential metabolite, tryptophane, in the reaction between the viruses T4 and T6 and their host. As to the function of the co-factor, it is not yet clear whether, as a cement substance, it acts in the specific combination between virus and host receptive spots or whether it acts as a sort of coenzyme in whose presence the virus particles, during their chance encounters with the host cells, are able to become attached to them and begin their parasitic activity. The high efficiency of Bz-3-methyltryptophane as a co-factor for the virus would suggest that the chain of essential reactions following virus adsorption does not involve the step which Bz-3-methyltryptophane blocks in the bacterial metabolism of tryptophane.

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THE LOCAL ANESTHETIC PROPERTIES OF ISONIPECAINE

WHILE investigating the actions of isonipecaine, 1-methyl-4-phenylpiperidine-4-carboxylic acid ethyl ester hydrochloride, it was noted that the compound produced pronounced corneal anesthesia when it was applied to the rabbit eye. Although isonipecaine has been reported[1, 2] to abolish the wink reflex, the effect was produced after systemic administration. The

[8] W. G. Gordon and R. W. Jackson, *Jour. Biol. Chem.*, 110: 151, 1935.

[1] O. Schauman, *Arch. f. Exper. Path. u. Pharmakol.*, 196: 109, 1940.

[2] R. C. Batterman, *Arch. Int. Med.*, 71: 345, 1943.