Divergent views have been expressed concerning urea-sulfonamide mixtures,[2,3] largely as a result of differences in technical methods and in interpretation. In the opinion of the present author true potentiation can be best demonstrated when the following conditions are fulfilled: (1) The medium should support luxuriant growth of the organisms, (2) measurements of bacteriostasis should be made frequently during the phase of active growth, and (3) concentrations of the bacteriostatic agents should not be so great in relation to the size of the inoculum that, either separately or in combination, active growth of the bacteria is prevented. The last point is especially important and requires further elucidation. When the bacteriostatic action of the mixture is too strong good growth of the organism never occurs; the effect is that of rendering the medium unsatisfactory. Under these circumstances valid comparisons cannot be made with experiments in which there is good initial bacterial growth. Superficially there may appear to be true potentiation, but actually there is not. This important fact, namely, that in experiments such as these bacteria must have certain minimal conditions favoring good growth, has been previously emphasized.[4] It is believed that such pitfalls and artefacts can best be avoided by adhering to the conditions listed above. If true potentiation exists, bacteriostasis of mixtures should be greater than merely the additive effects of the separate constituents when there is active growth of all the organisms. It is clear from Fig. 1 that, under these conditions, there is no actual synergism or potentiation; the effects are merely additive.

The potential clinical significance of these observations is difficult to predict. However, in seriously ill patients who are not responding well to penicillin it would seem not unreasonable to add the effect of another bacteriostatic agent such as sulfadiazine.

Summary. *In vitro,* sulfonamide-penicillin and urea-penicillin mixtures have been shown to produce greater bacteriostasis than penicillin alone with the *Staphylococcus aureus* and hemolytic streptococcus. This increased inhibition represents the additive effects of the separate bacteriostatic agents; there is no actual potentiation of penicillin action.

[2] Tsuchiya, H. M., Tenenberg, D. J., Clark, W. G., and Strakosch, E. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 262.

[3] Lee, S. W., Epstein, J. A., and Foley, E. J., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 105.

[4] Bloomfield, A. L., and Felty, A. R., *J. Exp. Med.*, 1924, **39**, 367.

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Folic Acid and Biotin Synthesis by Sulfonamide-sensitive and Sulfonamide-resistant Strains of *Escherichia coli.*

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When certain poorly absorbed sulfonamides, such as succinylsulfathiazole or sulfaguanidine, are incorporated in highly purified diets and fed to rats, bacteriological studies show the total number of organisms in the feces of such rats to be unaffected by the presence of the sulfonamide, although certain types of bacteria, such as the coliform organisms, may diminish or even disappear.[1] The feeding of such sulfonamide rations to weanling rats also results in the production of a nutritional deficiency[2,3] which may be corrected by feed-

[1] Gant, O. K., Ransone, B., McCoy, E., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 276.

[2] Welch, A. D., *Federation Proc.*, 1942, **1**, 171.

[3] Black, S., Overman, R. S., Elvehjem, C. A., and Link, K., *J. Biol. Chem.*, 1942, **145**, 137.

ing the animals biotin and folic acid.[4]

Black, McKibbin, and Elvehjem[5] originally postulated that such sulfonamides owe their ability to induce nutritional deficiencies to a depletion in the intestine of organisms capable of synthesizing B vitamins. More recently, however, Gant, Ransone, McCoy and Elvehjem[1] suggested that strains of *Escherichia coli* in the rat intestine may become resistant to the action of succinylsulfathiazole and that such resistant strains have probably lost much of their ability to synthesize growth factors such as biotin and folic acid.

We have studied the *in vitro* production of biotin and folic acid by *E. coli* to determine whether the state of sulfonamide resistance of the organism affected its ability to synthesize these vitamins.

Procedure. Several attempts to isolate a sulfonamide-resistant form of *E. coli* from the feces of rats which had been fed a purified diet containing 2% succinylsulfathiazole for at least three weeks proved unsuccessful. Therefore a laboratory strain of *E. coli* was treated in the following manner. From our original culture of the organism two strains were carried. One strain, designated "Normal," was transferred daily in a medium of the following composition: bacto-peptone, 1.0%; NaCl, 0.6%; glucose, 0.2%; K_2HPO_4, 0.1%. The other strain was carried in the bacto-peptone medium containing amounts of sulfanilamide that were gradually increased until the daily transfers could be made in medium containing M/100 drug. This strain was then called "Resistant."

The procedure for determining the amounts of biotin and folic acid synthesized by the "Normal" and "Resistant" strains was as follows: Twenty ml quantities of the bacto-peptone medium containing varying amounts of sulfanilamide were placed in 125 ml Erlenmeyer flasks and were sterilized by autoclaving at 12 lb pressure for 5 minutes. One ml of a 24-hour culture of the "Normal" strain in bacto-peptone medium or one ml of a 24-hour culture of the "Resistant" strain in bacto-peptone medium containing M/100 sulfanilamide was used as inoculum for each flask. The flasks were incubated 20 hours at 37°C, turbidities were determined, and the cultures were autoclaved to stop growth.

In the determination of folic acid the cultures were assayed both before and after a 24-hour period of takadiastase digestion. The assay organism was *Streptococcus fecalis* (*Streptococcus lactis R*), using the method described by Mitchell and Snell.[6] The folic acid standard was a solution of crystalline *Lactobacillus casei* factor kindly provided by the Lederle Laboratories.

In preparation for the biotin assays one ml of culture was autoclaved in the presence of one ml of 6N H_2SO_4 for one hour at 15 lb pressure. The assay organism was *L. casei e,* used according to the method of Landy and Dicken.[7] A solution of crystalline biotin served as a standard.

Turbidities of the cultures were read in a Klett-Summerson photoelectric colorimeter with a 540 mμ filter. Assay media contained 5 mg of *p*-aminobenzoic acid in every 100 ml. Adequate controls were run to correct for the amounts of folic acid and biotin present in the uninoculated growth medium. It was determined repeatedly that the amount of sulfanilamide in the samples had no inhibitory effect on the assay organisms.

Results. The results of 2 experiments are given in Table I. These assays indicate that the amount of folic acid produced in a culture was determined by the presence of sulfonamide in the growth medium, and not by the state of sulfonamide-resistance of the organism. That the amount of folic acid found in a given culture was not a function of the number of bacteria present was most strikingly demonstrated by the data given in Table I for the "Resistant" strain. It will be noted that this strain showed higher bacterial densities in the presence of the drug than in its absence. Folic acid synthesis, however, decreased as the concentration of sulfanilamide

[4] Welch, A. D., and Wright, L. D., *J. Nutrition,* 1943, **25**, 555.

[5] Black, S., McKibbin, J. M., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 308.

[6] Mitchell, H. K., and Snell, E. E., *The University of Texas Publication,* 1941, **4137**, 36.

[7] Landy, M., and Dicken, D. M., *J. Lab. and Clin. Med.,* 1942, **27**, 1086.

TABLE I.
Effect of Added Sulfonamide on Production of Folic Acid and Biotin by "Normal" and Sulfonamide "Resistant" Strains Developed from a Single Culture of *E. coli.*

Exp. No.	Strain	Molarity of sulfanilamide in growth medium	Turbidity after 20 hrs growth	Folic acid, γ/ml		Biotin γ/ml
				Untreated culture	Takadiastase treated culture	
I.	"Normal"	0	390	.018	.013	.0051
		M/100	223	.0002	.0000	.0015
		M/200	286	.0007	.0001	.0028
		M/400	315	.0012	.0007	.0030
	"Resistant"	0	330	.024	.020	.0032
		M/100	340	.0019	.0008	.0031
		M/200	350	.0083	.0059	.0040
		M/400	345	.018	.0080	.0044
II.	"Normal"	0	377	.015	.015	.0054
		M/100	210	.0000	.0004	.0011
		M/200	272	.0005	.0005	.0023
		M/400	286	.0012	.0008	.0032
	"Resistant"	0	280	.023	.023	.0037
		M/100	330	.0017	.0012	.0034
		M/200	345	.0075	.0071	.0041
		M/400	330	.015	.010	.0039

increased. Takadiastase digestion did not release extra folic acid from the culture but often decreased the amount of this vitamin that could be demonstrated by *S. fecalis* assay. This was particularly shown in the data of Experiment I.

Under the conditions of these experiments the amounts of biotin found in the cultures were not greatly influenced by the presence of sulfanilamide in the growth medium, but often varied with, and might possibly be a function of, the number of organisms present (turbidity).

Discussion. If these *in vitro* results are an indication of the *in vivo* activity of organisms in the intestinal tract of animals receiving a purified diet containing a poorly absorbed sulfonamide, it would seem that the presence of the sulfonamide in the intestine, and not the state of sulfonamide-resistance nor the total number of vitamin-synthesizing organisms present, influences the bacterial synthesis of growth factors and, thereby, the nutritional state of such animals.

Summary. Less folic acid was synthesized *in vitro* by both a sulfonamide-sensitive and by a sulfonamide-resistant strain of *Escherichia coli* when these organisms were grown in the presence of sulfanilamide than when they were grown in the same medium not containing the drug. The amount of biotin synthesized was not greatly affected by the presence of the drug in the growth medium.