TABLE I. Effect of Aureomycin on BSP Excretion and Fat Content of Liver in Normal Rats, Dogs, and Human Subjects.

Subject	No.	Dose/ day	Route of administration	Treatment (days)	Increase in BSP retention	Increase in liver fat histologically	Liver fat % by wt chemical analysis
Dogs	4	1 g	Oral	30	0	0	—
Rats	6	0	Saline IP	28	—	Control	—
	6	2 mg	IP	28	—	0	—
	6	5	"	28	—	0	—
	6	10	"	28	—	+, —	—
	25	0*	Oral	30, 60, 70†	—	Control	13.9 ± 1.1‡
	25	25	"	30, 60, 70†	—	0	13.8 ± 1.5‡
Humans	3	2 g	"	30	0	—	—

* Control animals pair-fed.
† 5 animals of each group sacrificed at 30 days, 15 at 60 days, 5 at 70 days.
‡ Mean ± std dev.

addition, cephalin cholesterol flocculation(9) and gamma globulin turbidity(10) tests were done on humans.

Results. (Table I). There were no significant changes in liver fat of dogs or rats as measured histologically and/or chemically after aureomycin administration for as long as 70 days. Two g aureomycin taken orally by three healthy young men for 30 days produced no changes in liver function tests in 2 subjects. One individual showed a change in bromsulphthalein retention from control values of 2% to 6% during therapy. This was not considered significant. There were no changes in the other tests done in this subject.

Comment. Dowling, *et al.*(11) observed increased liver fat and even death in some animals treated with large amounts of aureomycin. György, *et al.*,(12) has demonstrated that aureomycin actually prevents fatty liver and prolongs the survival of rats fed diets low in choline or methionine. On the other hand, clinical studies(1,2) indicate that some patients with hepatic disease or serious infections develop fatty liver after aureomycin administration, despite adequate diets. It is the purpose of this report to point out that these various effects were not obtained in normal dogs and rats maintained on adequate diets with levels of aureomycin dosage approximating the therapeutic range, and that no liver function test abnormalities developed in normal humans.

9. Hanger, F. M., *J. Clin. Invest.*, 1939, v18, 261.
10. DeLaHuerga, J., and Popper, H., *J. Clin. and Lab. Med.*, 1950, v35, 459.
11. Dowling, H. F., *et al.*, Personal communication.
12. György, P., Stokes, J., and Goldblatt, H., *Tr. Am. Assn. Phys.*, In press.

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Inhibition of Pantothenate Synthesis by Streptomycin.* (18813)

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During the past few years the mode of action of streptomycin has been studied by several groups. Thus, Umbreit(1), and Oginsky *et al.*(2) reported that streptomycin specifically inhibits an oxidative reaction, apparently the "oxalacetate-pyruvate" conden-

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1. Umbreit, W. W., *J. Biol. Chem.*, 1949, v177, 703.
2. Oginsky, E. L., Smith, P. H., and Umbreit, W. W., *J. Bact.*, 1949, v58, 747.

TABLE I. Growth Response of *S. fragilis* to β-alanine, Pantoyl Lactone Plus β-alanine, and Pantothenic Acid in the Presence and Absence of Streptomycin.

β-alanine (μg)	Pantoyl lactone† (μg)	Pantothenic acid (μg)	Concentration of streptomycin (mg) 0	.5	.75	1	2
0	0	0	20*	25	20	34	30
1	0	0	27	24	20	30	29
2	0	0	44	56	32	54	30
3	0	0	90	108	70	70	42
4	0	0	174	198	101	78	56
5	0	0	263	232	131	80	70
6	0	0	284	260	131	85	72
0	0	.025	31			32	34
0	0	.05	52			38	40
0	0	.075	78			63	67
0	0	.1	137			132	120
0	0	.25	200			240	219
0	0	.5	262			260	257
0	0	5	280			274	261
1	1	0	34			32	41
2	2	0	48			32	47
3	3	0	112			38	54
4	4	0	180			52	60
5	5	0	270			68	78
6	6	0	280			72	70

* Figures given are turbidity readings after 24 hr growth at 30°C. Concentrations are for 5.5 ml culture medium.

† Kindly supplied by Merck and Co., Rahway, N. J.

sation, which prevents a variety of substances from entering the terminal respiration system. Fitzgerald and Bernheim(3) presented data suggesting that this antibiotic inhibits the formation of adaptive enzymes, specifically the adaptive formation of a benzoic acid oxidase in *Mycobacterium lacticola*. More recently, Zeller *et al.*(4) showed that streptomycin inhibited the diamine oxidase reaction of *Mycobacterium smegmatis*, while Barkulis (5) reported that dihydrostreptomycin inhibits the formation of acetate and formate and metabolic CO_2 under anaerobic conditions in *Escherichia coli*.

The present paper is concerned with the synthesis of pantothenic acid by yeast, and the apparent inhibition of this synthesis by streptomycin.

Materials and methods. The organism employed was *Saccharomyces fragilis* (ATCC No. 2360). The response to *beta*-alanine and pantothenic acid was determined using the medium of Billen and Lichstein(6) modified so that it contained 1% acid-hydrolyzed vitamin-free casein (Nutritional Biochemicals Corp.). The techniques for assay were those already described(6) except that a total volume of 5.5 ml was employed. Streptomycin (calcium chloride complex-Merck or streptomycin sulfate-Squibb) was added aseptically to the medium in the desired concentrations. The assay tubes were incubated at 30°C, and growth determined after 24 hours by turbidity readings in a Klett-Summerson photoelectric colorimeter.

Results. It may be noted from the data given in Table I that streptomycin exerts a marked inhibitory effect on the growth of *S. fragilis* when this organism is grown in a synthetic medium containing *beta*-alanine as the precursor for pantothenate. In contrast, however, no effect on growth is obtained when streptomycin is incorporated into media containing preformed pantothenic acid. The concentration of streptomycin required is probably a reflection of the general resistance of yeast cells to this antibiotic and is possibly due to the relative impermeability of the cell to this compound. The data are repre-

3. Fitzgerald, R. J., and Bernheim, F., *J. Bact.*, 1948, v55, 765.

4. Zeller, E. A., Owen, C. A., Jr., and Karlson, A. G., *J. Biol. Chem.*, 1951, v188, 623.

5. Barkulis, I. L., *J. Bact.*, 1951, v61, 375.

6. Billen, D., and Lichstein, H. C., *J. Bact.*, 1949, v58, 215.

sentative of several experiments, and suggest that this antibiotic may inhibit the synthesis of pantothenate from *beta*-alanine.

Although it is probable that sufficient pantoyl lactone was synthesized by this organism, since growth proceeded normally when *beta*-alanine was the only addition to the medium, it was considered advisable to test the effect of streptomycin on the growth of this organism in the presence of both portions of the pantothenic acid molecule, as well as *beta*-alanine and pantothenic acid separately. Pantoyl lactone itself will not support the growth of this organism. The results of such experiments (Table I) suggest that the site of action of streptomycin in this system may be the inhibition of the coupling of *beta*-alanine and pantoyl lactone to form pantothenic acid. Although this conclusion appears reasonable on the basis of the growth studies presented, other possibilities exist. No definite conclusion can therefore be made until similar studies are carried out with resting cell suspensions or cell-free systems.

Summary. The results of studies on the growth response of *S. fragilis* to pantothenic acid and its precursors, *beta*-alanine and pantoyl lactone, in the presence of streptomycin, are interpreted to suggest that this antibiotic somehow interferes with the synthesis of pantothenate.

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Acquired Resistance to the Shwartzman Phenomenon.* (18814)

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Although previous investigations of the effect of repeatedly eliciting the Shwartzman reaction had been somewhat contradictory, Beeson(1) showed clearly that if the reaction is produced at 2-day intervals, successive lesions diminish in intensity and animals become completely non-reactive. This state of resistance was abolished by the injection of thorotrast. The experiments reported here were designed to further elucidate this type of resistance to the Shwartzman phenomenon.

Experimental methods. White male rabbits of mixed breed weighing 2000 to 3000 g were used. The materials employed to elicit the reaction were the P-25 polysaccharide from *Serratia marcescens* (obtained from Dr. M. J. Shear) and meningococcus culture filtrate (prepared by Dr. B. Black-Schaffer). The dosage of P-25 was 0.05 mg in 0.5 ml physiologic saline intracutaneously for preparation and 0.1 mg in 1.0 ml intravenously. In some experiments these amounts were doubled, the injected volume remaining the same. Meningococcus filtrate was diluted 1:10 with physiologic saline; 0.2 ml of this material was used for skin preparation and 0.5 ml intravenously. With this dosage about 80% of animals tested showed typical hemorrhagic lesions. The intravenous "provocative" injection was given 24 hours after skin preparation and the resulting skin lesions were noted and recorded 24 hours later. The presence of hemorrhage was the only criterion used for recording the reaction as positive. In addition to the above materials, purified typhoid antigen (obtained from Dr. H. R. Morgan), which is also capable of eliciting the Shwartzman reaction was used. When multiple skin injections were given, the first was made in the right upper quadrant of the abdomen, the second in the left upper quadrant, the third on the right just caudal to the first, etc., alternating sides of the abdomen and moving the sites posteriorly. Hair was removed with electric clippers immediately prior to injection. For passive transfer experiments, blood was ob-

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1. Beeson, P. B., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 146.