in the medium and to the salt concentration.

3. The effect of salt is exerted not upon the streptomycin itself but upon the medium, which in turn influences the growth of the organism.

16277

Effect of Organic Acids on Streptomycin Activity.*†

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The antibacterial potency of streptomycin is still largely measured by its inhibiting effect upon the growth of certain bacteria in artificial culture media, under given conditions of cultivation. The activity of the antibiotic thus measured is influenced by a great many factors. Chief among these is the presence of certain organic and inorganic constituents in the medium,[1] the presence of certain reducing substances,[2,3] the nature of the test organism, its age and number of viable cells used.[4] In addition to these, the presence of certain organic acids that may be formed as intermediary metabolic products in the nutrition of various bacteria may also affect the activity of streptomycin. A study of the last phenomenon forms the subject of this report.

Escherichia coli ATTC 9637 was used as the test organism. Its growth in various media was determined by means of a Cenco-Sheard-Sanford Photelometer. Immediately after inoculation, the culture tubes gave a reading of zero turbidity. The final turbidity was expressed in terms of per cent of light absorption.

The organic acids used in these studies were neutralized to pH 7.0, sterilized by filtration through Mandler candles, and added to the medium to give a final concentration of 1%. The streptomycin solution was sterilized by heating at 60°C for 30 minutes. The basic nutrient broth used in these experiments contained 0.5% peptone and 0.3% meat extract.

The influence of different organic acids as compared to that of sugars and glycerol upon streptomycin activity was at first determined (Table I). The growth of *E. coli* in nutrient broth is usually inhibited by 1 μg of streptomycin per ml. When pyruvic or fumaric

TABLE I.
Effect of Various Carbon Sources in Medium upon Streptomycin Activity on *E. coli*.

Carbon source 1%	Turbidity* 4 hr Streptomycin, 10 μg/ml 0	+	21 hr Streptomycin, 10 μg/ml 0	+
Glucose	—	—	41	0
Lactose	14	0	36	0
Pyruvic acid	23	13	67	31
Fumaric acid	14	12	53	30
Formic acid	0	0	15	12
Succinic acid	8	6	30	7
Glycerol	8	0	42	0
Maleic acid	10	6	40	12
Malonic acid	8	6	31	6
Glycerophosphoric acid	7	0	33	0
Acetic acid	6	0	34	0
Propionic acid	5	0	30	0
Lactic acid	9	0	52	0

* Absorption of light in per cent.

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1 Green, S. R., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 281.

2 Geiger, W. B., Green, S. R., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 187.

3 Donovick, R., and Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 224.

4 Lenert, T. K., and Hobby, G. L., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 235, 242.

TABLE II.
Effect of Different Concentrations of Streptomycin upon Growth of *E. coli* in Presence of Pyruvic or Fumaric Acids.

Carbon source 1%	Streptomycin, μg/ml	Turbidity			
		5 hr	8 hr	17 hr	22 hr
Pyruvic acid	0	14	19	50	48
	10	7	14	33	34
	25	2	5	23	27
	50	0	0	0	2
	100	0	0	0	0
Fumaric acid	0	10	25	49	49
	10	9	20	40	41
	25	6	16	29	32
	50	1	3	15	16
	100	0	0	0	0

TABLE III.
Influence of Nitrogen Sources upon Effect of Organic Acids and Other Carbon Compounds upon Streptomycin Activity.*

Additional material	Casein hydrolysate		Asparagine Streptomycin, 10 μg/ml		Tryptone	
	0	+	0	+	0	+
None	20	0	2	0	22	0
Glucose	38	0	2	0	39	0
Lactose	33	0	0	0	30	0
Formic acid	4	3	4	0	13	6
Maleic acid	29	22	5	5	21	10
Malonic acid	44	10	11	0	21	4
Malic acid	51	15	16	0	40	18
Succinic acid	38	10	12	0	30	10
Pyruvic acid	48	37	50	2	42	40
Glycerol	37	0	2	0	29	0
Fumaric acid	35	22	17	2	36	19
Lactic acid	57	0	9	0	36	0

* *E. coli* used as test organism; incubation 20 hours at 28°C.

acid was added to the medium good growth was obtained even in the presence of 10 μg/ml of the antibiotic. Succinic, formic, maleic, and malonic acids also exerted a certain degree of protection upon the bacteria against the antibacterial action of streptomycin. Lactose, glycerol, sodium glycerophosphate, lactate, and glucose had, however, no effect upon streptomycin activity; the same was true of propionic and acetic acids.

The pyruvate and fumarate media in which *E. coli* grew in the presence of streptomycin were tested, by the agar cup method, for streptomycin concentration. The antibiotic was not reduced in potency. The possible development of bacterial strains resistant to streptomycin was also determined; the culture was found to remain sensitive to 2 μg/ml of streptomycin.

A study was made next of the minimum concentration of streptomycin necessary to inhibit the growth of *E. coli* in the presence of pyruvate and fumarate. Even in the presence of 50 μg/ml of streptomycin good growth was obtained in the peptone broth to which fumarate was added; there was no growth, however, with 100 μg/ml streptomycin (Table II). The effect of pyruvate was similar to that of fumarate, although quantitatively somewhat different. When the concentration of the fumarate was increased to 3%, growth occurred even in the presence of 150 μg/ml of streptomycin but none with 200 μg/ml.

The addition of organic acids and sugars to tryptone or casein hydrolysate broth gave results similar to those obtained with the peptone broth (Table III). When asparagine

was used as a source of nitrogen, slight growth of *E. coli* occurred in the presence of streptomycin with maleic acid, and a trace of growth with pyruvic and fumaric acids; the other carbon sources did not antagonize the action of streptomycin upon the growth of *E. coli*. This indicated the possibility that the complex nitrogenous materials contained some substance which was necessary for the utilization of the pyruvate and fumarate in the presence of streptomycin; this substance could not be derived from asparagine.

In an effort to elucidate the possible nature of the substance which favored the neutralizing effect of pyruvate and which was absent in asparagine, yeast extract and a vitamin mixture containing B_1, B_2, B_6, calcium pantothenate, folic acid, nicotinamide, para-aminobenzoic acid, and cocarboxylase were added to asparagine-pyruvate broth. Only the yeast extract proved effective in allowing the growth of *E. coli* in the presence of streptomycin (Table IV).

TABLE IV.
Effect of B Vitamins and Yeast Extract upon Antibacterial Activity of Streptomycin in Asparagine-Pyruvate Broth.
Asparagine 2.5 mg/ml, pyruvate 10 mg/ml.

Supplementary additions to broth		Turbidity after 25 hrs Streptomycin, 10 μg/ml	
	Mg/ml	0	+
Broth alone*		13	0
Pyruvate control		28	28
Yeast extract	2.5	51	17
" "	1.25	50	6
" "	0.63	52	8
Vitamin mixture		8	0

* No pyruvate.

The neutralizing effect of pyruvate and fumarate upon streptomycin was tested with other bacteria, including another strain of *E. coli* (No. 2), *Aerobacter aerogenes, Proteus vulgaris,* and *Staphylococcus aureus*. The action of pyruvate and fumarate upon streptomycin activity was found to vary with the organism (Table V). It was most marked with *P. vulgaris* and *E. coli,* and least with *A. aerogenes* and *S. aureus*. Similar variations were obtained with other organisms. *Mycobacterium tuberculosis* No. 607 showed some inhibition by fumarate with low concentrations of streptomycin and none with higher concentrations; the pyruvate showed no effect at all for this organism.

The results tend to show that certain organic acids have a definite neutralizing or antagonistic effect upon the antibacterial properties of streptomycin only as regards certain organisms. The salts of the dicarboxylic acids (fumaric, succinic, malic, and maleic), as well as pyruvic and formic acids, were most effective; they supported the growth of *E. coli* in the presence of bactericidal concentrations of streptomycin. The salts of acetic, lactic, and propionic acids, as well as sugars, such as glucose and lactose, did not influence the inhibiting action of streptomycin upon *E. coli*.

The presence of pyruvic and dicarboxylic acids does not result in the destruction of the antibiotic, since the concentration of streptomycin in the medium remained close to the theoretical. These organic acids do not favor the development of strains of bacteria resistant to the action of streptomycin. No irreversible combination is produced between the antibiotic and the organic acid. The only explanation for this effect may be looked for in the susceptibility of a specific enzyme system to the action of streptomycin, whereby it may be replaced, at least in the metabolism of certain bacteria, by another system in the presence of pyruvic or fumaric acid. That the effect of streptomycin upon susceptible bacteria may be due to interference with intermediary metabolism has been suggested.[6]

It may also be of interest to note here that the oxidation of benzoic acid by *Mycobacterium tuberculosis* No. 607 was inhibited by 10 μg/ml streptomycin, whereas even 100 μg had no effect upon this oxidation process by a resistant strain of this organism. On the other hand, 100 μg streptomycin had no effect upon the oxidation of pyruvic acid by the normal strain.[7]

The possibility that streptomycin interferes with the synthesis of amino acids, proteins, or carbohydrates and that the organic

5 Geiger, W. B., *Arch. Biochem.*, 1947,

6 Strauss, E., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 97.

7 Bernheim, F., and Fitzgerald, R. J., *Science,* 1947, **105**, 435.

TABLE V.
Effect of Pyruvate and Fumarate upon Antibacterial Activity of Streptomycin.
Incubation 17 hours at 28°C.

Carbon source	*E. coli*		*A. aerogenes*		*P. vulgaris*		*S. aureus*	
	0*	+	0	+	0	+	0	+
None	15	0	18	0	4	0	24	0
Pyruvic acid	36	29	40	4	36	26	44	13
Fumaric acid	40	23	46	6	47	42	22	6
Glucose	37	0	56	0	14	0	44	0

* 0 = no streptomycin; + = 4 μg/ml streptomycin.

acids may counteract the antibiotic effect by acting as essential metabolites in the synthesis of these compounds has been suggested.[5] Certain preliminary results obtained in oxidation experiments, by the use of the Thunberg and Warburg techniques, indicated that streptomycin does not affect metabolic reactions measured in terms of oxygen uptake or dehydrogenase activity; rather it appeared that streptomycin affects a synthetic process, and the effect on respiration is only a secondary phenomenon.

Summary. The addition of 1% pyruvate and fumarate to nutrient broth supported the growth of *E. coli* in the presence of 10 μg/ml of streptomycin. When the concentration of the acids was increased to 3%, growth took place even in the presence of 150 μg/ml of streptomycin. The streptomycin was not destroyed. The test bacteria growing in the presence of streptomycin and of the organic acids did not become resistant to streptomycin.

Salts of succinic, formic, malic, and maleic acids also exerted some antagonistic effect upon streptomycin. Lactose, as well as lactic, acetic, and propionic acids, glycerol, glycerophosphate, and glucose had no effect upon the growth-inhibiting action of streptomycin on *E. coli.*

Comparison of the antagonistic effect of pyruvic and fumaric acids against the streptomycin action upon various bacteria, showed considerable variation. *E. coli* and *P. vulgaris* were largely protected by the organic acids against the action of streptomycin, whereas *A. aerogenes* and *S. aureus* were only slightly affected.

16278

Urinary Excretion of 17-Ketosteroids by Normal Young Men During Starvation.*

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There are only a few reports indicating the effect of starvation on the urinary excretion of 17-ketosteroids. In most of these, owing

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