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Effect of Nutrients Upon Growth of Streptomycin-Sensitive, -Resistant- and -Dependent Strains of *Escherichia coli.**†

WARREN P. IVERSON AND SELMAN A. WAKSMAN.

From the Department of Microbiology, New Jersey Agricultural Experiment Station, Rutgers University.

Among the various constituents of the medium which influence the antibacterial potency of streptomycin, salt, glucose and certain organic acids were found[1,2] to be of special importance. The presence in nutrient broth of pyruvic and fumaric acids in 1% concentrations enabled *Escherichia coli* to grow in the presence of 10 μg/ml of streptomycin, whereas in the same medium free from these acids, the organism was inhibited even by 1 μg/ml of the antibiotic. With the isolation of streptomycin-dependent strains of this organism, it was considered of importance to determine how the growth of such dependent strains (*E. coli* ds) would be affected by the presence in the medium of those substances which normally favor growth of the original streptomycin-sensitive cultures (*E. coli* ss) in the presence of streptomycin.

Effect of NaCl upon the growth of E. coli ds. Varying amounts of NaCl, sterilized by autoclaving, and streptomycin pasteurized by heating for 30 minutes at 60°C, were added to sterile nutrient broth (0.5% peptone and 0.3% meat extract in distilled water) in tubes. These were inoculated with a suspension of *E. coli* ds grown on nutrient agar containing 10 μg/ml streptomycin. Each tube was thus inoculated with 44,000 viable cells. The cultures were incubated at 28°C and the amount of growth was measured by using a Cenco-Sheard-Sanford Photelometer type B2 with a red filter. Growth was expressed as the average turbidity (100–transmittancy) of three tubes.

E. coli ds did not grow in the media free from streptomycin; in the presence of streptomycin, however, good growth occurred (Table I). The presence and concentration of NaCl had an important effect. Without the salt, growth was poorer and was more sensitive to higher concentrations of streptomycin, so that 500 μg/ml was sufficient to inhibit completely the growth of the organism. With an increase in concentration of NaCl to 1%, there was a tendency for more rapid initial growth with increasing concentrations of streptomycin. In other words, the presence of NaCl exerts a favorable effect upon the growth of both *E. coli* ss and *E. coli* ds in the presence of streptomycin: The injurious effect of streptomycin upon *E. coli* ss is reduced by the presence of NaCl, and growth of *E. coli* ds is favored.

Influence of carbon sources upon the growth of E. coli *ds.* A study was made next of the effect of glucose and of salts of pyruvic and fumaric acids upon the growth of *E. coli* ds (Table II). The above nutrient broth (free from NaCl) was used. When no other carbon source was added, growth was rather limited; an increase in the concentration of streptomycin above 5 μg/ml had a somewhat delaying and even depressing effect upon the growth of the organism. When glucose was added to the broth there was a marked increase in the amount of growth produced; increasing the concentration of streptomycin above 5 μg/ml had again a delaying effect upon the rate of growth. Pyruvate and fumarate also exerted a highly favorable effect upon the growth of *E. coli* ds. This was especially true of the pyruvate; in this case, however, an increase in the concentration of the streptomycin above

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TABLE I.
Influence of NaCl on Growth of *E. coli* ds.

	No NaCl					0.4% NaCl					1.0% NaCl				
	Concentration of streptomycin, μg/ml														
Hrs of incubation	0	5	25	100	500	0	5	25	100	500	0	5	25	100	500
	Turbidity.														
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	7	0	0	0	0	11	10	4	0	0	0	10	7	1
25	0	13	2	0	0	0	15	14	13	0	0	1	14	19	12
43	0	18	13	0	0	0	18	19	19	5	0	11	20	19	16
91	0	18	16	9	0	0	26	24	24	20	0	14	28	26	24
139	0	15	13	12	0	0	25	22	21	16	0	17	21	19	18

TABLE II.
Influence of Carbon Sources upon the Growth of *E. coli* ds.

	No added carbon source				Glucose				Pyruvate				Fumarate			
	Concentration of streptomycin, μg/ml															
Hrs of incubation	0	5	15	25	0	5	15	25	0	5	15	25	0	5	15	25
	Turbidity.															
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	1	1	0	0	3	0	0	0	0	2	2	0	0	0	2
24	0	10	4	0	0	23	14	5	0	2	19	23	0	1	12	17
41	0	18	18	13	0	36	38	35	0	19	38	42	0	11	26	25
95	0	21	22	18	0	44	45	43	0	27	50	52	0	22	38	40
119	0	20	21	16	0	48	49	46	0	33	52	53	0	23	40	43

TABLE III.
Influence of Carbon Sources in a Synthetic Medium upon the Growth of *E. coli* ss in Presence of Streptomycin.

	Glucose		Pyruvate		Fumarate	
	Concentration of streptomycin, μg/ml					
Incubation, hr	0	5	0	5	0	5
			Turbidity			
24	0	0	0	0	0	0
48	0	0	10	0	4	0
72	2	0	14	0	9	0
96	21	0	11	0	9	0
120	25	0	14	0	13	0
142	26	0	16	0	15	0
169	30	0	20	0	18	0
193	32	0	21	0	23	0
265	35	0	27	0	28	0
336	36	0	30	0	33	0
528	39	0	43	0	42	0

5 μg/ml not only had no delaying effect but proved to be more favorable to growth. These results thus tend to prove that pyruvate and fumarate favor the growth of both *E. coli* ss and *E. coli* ds, in the presence of streptomycin.

Use of synthetic media for studying the effect of carbon sources upon streptomycin activity on various E. coli strains. In order to eliminate the interfering action of peptone and meat extract upon the effect of supplementary addition of carbon sources, a synthetic (Koser's) medium was used. This medium consisted of 1 g $(NH_4)H_2PO_4$, 1 g K_2HPO_4, 0.2 mg $MgSO_4 \cdot 7H_2O$, 5 g NaCl, 1000 ml distilled water, and was adjusted to pH 7.3. The carbon sources, previously sterilized by passage through a Seitz filter, were added to make a final concentration of 0.2%. Several strains of *E. coli* of varying sensitivity to streptomycin were used in these

TABLE IV.
Influence of Carbon Sources in a Synthetic Medium upon the Growth of *E. coli* ds in Presence of Streptomycin.

Incubation,hr	Glucose				Pyruvate				Fumarate			
	Concentration of streptomycin, μg/ml											
	5	50	500	1000	5	50	500	1000	5	50	500	1000
	Turbidity											
24	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	1	1*	0	0	0	0	0	0
66	0	0	0	0	4	5	0	0	0	2†	4	1†
90	0	0	0	0	13	13	0	0	1*	5	9	5
118	0	0	0	0	16	15	10	0	3	10	15	14
138	0	0	1*	0	18	16	8	0	5	13	19	18
164	0	13	10	0	19	18	17	4†	7	16	22	22
193	2*	23	20	0	22	20	19	16†	10	20	23	23
261	23*	28	22	21*	24	22	21	10*	14	27	23	21
384	24	32	26	23	26	25	24	21	22	26	16	15
648	30	36	30	30	38	37	18	15	32	30	16	15

Measurements reported are averages of 3 tubes, except those indicated by * where growth occurred in 2 tubes only and † with growth in one tube only. No growth in media free from streptomycin.

TABLE V.
Influence of Carbon Sources in a Synthetic Medium upon the Growth of *E. coli* rs‡ in Presence of Streptomycin.

Incubation, hr	Glucose					Pyruvate					Fumarate				
	Concentration of streptomycin, μg/ml														
	0	5	50	500	1000	0	5	50	500	1000	0	5	50	500	1000
	Turbidity														
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	1*	1	0	0	1†	1*	1*	0	0
71	0	0	0	0	0	11	16	16	4	0	5	7	8	7	2*
96	0	0	0	0	0	19	18	20	16	0	10	11	12	13	8
120	0	0	0	0	0	22	20	23	18	7	15	14	16	17	16
144	13†	0	0	0	0	24	22	26	23	17	18	18	22	22	21
168	16	9*	6†	0	0	28	24	27	25	21	21	21	24	24	23
240	24	25	22	0	26†	33	29	32	29	26	27	27	32	28	24
336	28	31	27	26†	24	36	32	34	30	26	29	25	25	19	15
528	31	35	31	34†	31†	44	40	41	26	16	34	30	26	19	16

‡ This strain was obtained from a culture of *E. coli* ss growing in presence of streptomycin.

experiments, namely the streptomycin-sensitive (ss), streptomycin-resistant (rs) and streptomycin-dependent (ds).

Growth of *E. coli* ss was inhibited by 5 μg/ml of streptomycin, as shown in Table III. The presence of pyruvate and fumarate in the medium did not overcome the inhibiting effect of the antibiotic in the above concentration. *E. coli* ds grew well in all tubes containing streptomycin, varying in concentration between 5 to 1000 μg/ml (Table IV). With the higher amounts of streptomycin, growth with glucose was somewhat better than with pyruvate and fumarate. As one would expect, no growth occurred in the media free from streptomycin.

A streptomycin-resistant strain of *E. coli* (rs) was obtained by allowing the parent culture to grow in nutrient broth containing 1 μg/ml of streptomycin, plating out the culture at the end of a month's incubation, using agar media containing 1000 μg/ml streptomycin, and isolating one of the colonies. This culture of *E. coli* rs grew best with pyruvate as a source of carbon, both in the absence and in the presence of streptomycin (Table V). An increase in concentration of the antibiotic resulted in a delaying effect upon growth of the culture in the presence of glucose, and to a lesser extent in the presence of the salts of the organic acids.

A second resistant strain was obtained by plating out a 28 hour old nutrient broth culture of the parent sensitive strain of *E. coli*

TABLE VI.
Growth of *E. coli* rs* in a Synthetic Medium Containing Different Carbon Sources and Various Concentrations of Streptomycin.

	Glucose			Pyruvate			Fumarate		
	Concentration of streptomycin, μg/ml								
Incubation, hr	0	5	500	0	5	500	0	5	500
	Turbidity								
98	0	0	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0	0	4
146	0	0	0	0	0	0	0	0	8
168	0	0	0	0	0	0	0	0	13
192	0	0	0	0	0	0	0	0	19
213	0	0	0	0	0	4	0	0	19
238	0	0	0	0	0	10	0	0	20
265	0	0	0	0	0	15	0	0	21
290	0	0	0	0	0	15	0	0	20
362	0	0	0	0	0	15	0	0	15
432	0	0	0	0	0	22	0	0	13
624	0	0	0	0	0	16	0	0	13

* This strain was isolated from the plating of *E. coli* ss in agar containing streptomycin (15 μg/ml).

using nutrient agar to which 15 μg/streptomycin had been added. Of the colonies which appeared on the plate, one was found to be resistant to streptomycin. Of the remaining ten, 5 were similar to the first in resistance to streptomycin (in other words, they were *E. coli* rs strains) and 5 were dependent on streptomycin (*E. coli* ds strains). The first colony gave rise to a culture which grew only in synthetic media in the presence of pyruvate or fumarate as a carbon source and with streptomycin only in concentrations of 500 μg/ml (Table VI); no growth took place in higher (1000 μg/ml) or lower (100 μg/ml) concentrations. When such growth in the pyruvate or fumarate tubes was streaked on plain nutrient agar or on agar containing 10 μg/ml of streptomycin, all grew on the streptomycin agar but not on the streptomycin-free agar. Two of the 3 pyruvate tubes also gave some growth on the plain agar. When this freshly isolated *E. coli* ds was inoculated into nutrient broth, allowed to grow for 4 days at 28°C, then plated out on plain agar and on streptomycin-containing (10 μg/ml) agar, 1 ml of the culture contained 137,000,000 sensitive cells and 470,000 dependent cells of *E. coli*. A streptomycin-resistant strain was thus transformed, by growing in a fumarate or pyruvate medium, first into a streptomycin-dependent and then into a streptomycin-sensitive strain.

A preliminary experiment in which various amino acids were added to the synthetic medium with glucose as a carbon source seemed to indicate that l-lysine was necessary for the growth of this resistant organism.

These results thus bring out emphatically the relationship between the nutrition of *E. coli* and its sensitivity or resistance to or dependence on streptomycin.

Summary. 1. Growth of a streptomycin-dependent strain of *E. coli* in nutrient broth was favored by the presence of sodium chloride.

2. Glucose, pyruvate, and fumarate, when added to nutrient broth minus sodium chloride, caused greater growth of the streptomycin-dependent strain. With nutrient broth alone and nutrient broth plus glucose, the greatest initial growth took place in the presence of 5 μg streptomycin/ml. Nutrient broth plus the sodium salts of pyruvate and fumarate, however, favored a greater initial growth in higher concentrations of streptomycin (25 μg/ml).

3. In a synthetic medium containing glucose, pyruvate and fumarate as carbon sources, the growth of the sensitive parent *E. coli* strain was inhibited by 5 μg/ml; the dependent strain grew in all concentrations of streptomycin from 5 to 1000 μg/ml, but not in media without streptomycin. One resistant strain grew in all concentrations of

streptomycin as well as in media without streptomycin, while a second resistant strain grew only in a streptomycin concentration of 500 μg/ml with pyruvate and fumarate as carbon sources.

4. This second streptomycin resistant strain was transformed into a streptomycin-dependent strain under the above conditions. The dependent strain so obtained remained dependent after several transfers on nutrient agar containing streptomycin, and in turn gave rise to streptomycin-sensitive cells when a large inoculum was placed in streptomycin-free nutrient broth.

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A Simple Technic for Counting Megakaryocytes.*

EDWIN E. OSGOOD.

From the Division of Experimental Medicine, University of Oregon Medical School.

Principle of method. Make a thick drop preparation of an accurately measured volume of marrow as in examination for malaria and count all the megakaryocytes in the preparation.

Method. From a well-shaken, evenly suspended marrow specimen,[1] fill a Sahli hemoglobin pipette to the 20 cmm mark and blow contents onto a clean slide to form a thick drop. Spread evenly with the pipette tip over an oval area about 1.5 × 3 cm. Usually several such thick drop slides are made so that additional slides are available for examination in case part of the drop is detached by too vigorous washing and so that, when the count is low, a statistically significant number of cells can be counted.[1] Place slides in an incubator overnight. The following morning, immerse the slides in a solution of 5% formalin in 1% acetic acid in a Coplin jar until the erythrocytes are lysed and the thick drop is grayish in color. Flood the laking solution off the slide with water and gently rinse with buffer-phosphate solution (pH 6.4).[2] Stain with Wright's stain alone for 2 to 3 minutes. Add buffer-phosphate solution and stain 60 to 90 minutes. Wash slide by holding horizontally under gently running water. Air dry. Spread a thin film of immersion oil over the smear and examine systematically, using a mechanical stage and 200 × magnification, preferably with an 8 mm objective which is not immersed in the oil. Count all megakaryocytes seen in the specimen and multiply by 50 to express as number per ml. If the total nucleated marrow cell count per cmm and the leukocytic-erythrocytic ratio are determined, the number of megakaryocytes per million nucleated marrow cells or per million nucleated erythrocytic cells may be calculated. Morphology is fairly well preserved and any cell can be examined in greater detail by switching to the oil immersion lens.

In previously reported methods a count was made of all megakaryocytes and all nucleated marrow cells[3] or of all megakaryocytes and all nucleated erythrocytic cells[4] in an area of arbitrarily determined size on a thin marrow smear and results expressed, respectively, as megakaryocytes per million nucleated marrow cells or per million nucleated erythrocytic cells. These methods have the disadvantages that they are extremely time-consuming and that such large cells are seldom distributed evenly and, indeed, are likely to be found in greatest numbers at the tails of the smear. The area selected for examination will thus

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