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Sulfanilamide Activity Against *Escherichia coli* Under Anaerobic Conditions.*

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The efficacy of sulfanilamide (SA) as an inhibitor of the aerobic growth of *Escherichia coli* in synthetic media and of *p*-aminobenzoic acid (PAB) as an anti-SA has been well established.[1,2,3] The studies of Broh-Kahn[4] and of Fox[5] raise some doubt as to the bacteriostatic activity of SA against *Esch. coli* under anaerobic conditions. The present study was carried out in an attempt to obtain more quantitative data on SA activity under anaerobic conditions of growth in a synthetic medium commonly employed in studies under aerobic conditions.

It was found (unpublished results) that high concentrations of SA markedly inhibit oxygen uptake of washed suspensions of *Esch. coli* (K-12) in the presence of sugars, amino acids, fatty acids, succinate, fumarate, lactate and pyruvate, the degree of inhibition depending to a considerable extent on the concentration of SA and of bacteria. PAB has little demonstrable anti-SA activity under these conditions and in relatively high concentrations by itself exerts an inhibitory action on respiration. PAB does effectively block SA inhibition of the aerobic growth and respiration of this strain of *Esch. coli* in the synthetic medium. SA has no appreciable effect on dehydrogenase activity with methylene blue as the hydrogen acceptor, even in saturated solutions of SA and after 2 to 5 hours' exposure of the organisms to the drug. Thus the inhibition of respiration produced by SA must occur after the initial activation of the substrate. If SA is an active depressant of respiration under anaerobic conditions, its point of attack may lie between the original dehydrogenase system and the final H-acceptor.

Experimental. The basal medium (Wood[3]) was diluted with an equal volume of sterile M/7.5 phosphate buffer of pH 7.2 (or a solution of Na bicarbonate) and inoculated with a previously centrifuged suspension of an 18-hour thioglycollate broth culture. Essentially similar results were obtained with inocula from cultures in the synthetic medium with the exception that a longer lag period of growth was observed. The inoculated medium was distributed in vented Warburg flasks containing measured volumes of SA and PAB solutions and the air in the flasks immediately displaced with O_2-free H_2 or N_2 or with a 95% H_2-5% CO_2 mixture when bicarbonate was employed as the buffer system. In a number of duplicate experiments a small particle of phosphorus was present in each flask to ensure complete removal of oxygen. However, no difference in results was observed in the presence or absence of phosphorus.

Attempts were made to determine the amounts of CO_2 and of H_2 produced during growth. However, the reduced CO_2-tension produced with KOH as the CO_2-absorbent markedly increases the lag period of growth of *Esch. coli* under anaerobic conditions, the amount of growth in 6 hours generally being 50 to 75% less than in the absence of KOH. The manometric results will therefore be reported as CO_2. Growth was estimated from turbidimetric measurements in a Klett-Summerson photoelectric colorimeter. All experiments were carried out at 37°C.

Results. A comparison of the activity of SA under aerobic and anaerobic conditions is presented in Table I. A heavier inoculum (3x) was employed in the anaerobic culture and growth was allowed to proceed 90 minutes longer.

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1 Kohn, H. I., and Harris, J. S., *J. Pharm.*, 1941, **73**, 383.

2 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

3 Wood, W. B., Jr., *J. Exp. Med.*, 1942, **75**, 369.

4 Broh-Kahn, R. H., *Science*, 1939, **90**, 543.

5 Fox, C. L., Jr., *Science*, 1940, **91**, 477.

TABLE I.

Influence of SA on Aerobic and Anaerobic (in H_2) Growth and Respiration of *Esch. coli.*

		Mm³ gas			Bact./ml	
		O_2	as CO_2	% control	× 10⁸	% control
Control	Aerobic	161			19.8	
	Anaerobic		168		19.5	
SA M/12,800	Aerobic	50		31.1	5.0	25.3
	Anaerobic		62	36.9	13.5	69.2
SA M/25,600	Aerobic	69		42.9	9.0	45.0
	Anaerobic		118	70.3	16.5	84.6
SA M/51,200	Aerobic	95		59.0	11.5	58.1
	Anaerobic		136	80.9	19.5	100

TABLE II.

Influence of M/1600 SA on Anaerobic (in N_2) Respiration and Growth of *Esch. coli* per ml of Synthetic Medium.

	No. bact.	Total Mm³ CO_2	Mm³ CO_2/cell/hr
Control			
0 hr	2.42 × 10⁸	—	—
2.5 "	3.85 "	20	2.6 × 10⁻⁸
4.5 "	16.83 "	132	6.3 "
5.5 "	34.10 "	545	16.9 "
+ SA M/1600			
0 hr	2.42 × 10⁸	—	—
2.5 "	3.85 "	18	2.4 × 10⁻⁸
4.5 "	6.82 "	57	3.8 "
5.5 "	10.12 "	101	5.2 "
% of control	29.7	18.5	

Table I shows that, under our experimental conditions, SA inhibits respiration and growth to a similar extent under aerobic or anaerobic conditions. With the heavier inocula necessitated by the slower rate of growth under anaerobic conditions, it is necessary to use somewhat higher concentrations of SA than under aerobic conditions. SA also exerts its inhibitory action on growth in the bicarbonate buffered as well as in the phosphate buffered medium. In the bicarbonate medium CO_2 production appears to be inhibited to a greater extent than acid production. It has been observed (Sevag,[6] unpublished results) that the carboxylase system is quite sensitive to the sulfa-drugs. However, the studies on SA activity in the bicarbonate medium are complicated by the marked changes observed in pH due to acid production with consequent decomposition of the bicarbonate. SA activity is of the same magnitude in an atmosphere of H_2 as of N_2 with the possible exception that H_2 itself may exert a slight inhibitory action on gas production (Woods[7]).

In Table II results are presented showing the influence of SA on growth and respiration during the early stages of growth in the synthetic medium under anaerobic conditions.

It is apparent that the rates of respiration and of growth are inhibited by SA to a similar extent during anaerobic growth in the synthetic medium. Also, a lag period in SA inhibition is observed under anaerobic conditions similar to that usually observed in aerobic cultures. It must be borne in mind that this lag period of SA inhibition of respiration is not observed with washed suspensions.

The inhibitory effect of SA can be prevented by the addition of PAB to the culture medium.

[6] Sevag, M. G., Shelburne, M., and Mudd, S., *J. Gen. Physiol.*, 1942, **25**, 805.

[7] Woods, D. D., *Biochem. J.*, 1936, **30**, 515.

In a typical experiment 17 mm^3 of gas was produced in 7.5 hours in the presence of M/1600 SA as compared with 190 in the control and 172 in M/1600 SA + M/200,000 PAB. The corresponding growth figures are 5.2, 25.3 and 25.4 bacteria $\times 10^8$ per ml respectively.

Conclusions. The results of this study show that SA inhibits the respiration and growth of *Esch. coli* in a synthetic medium with glucose as the source of energy under either aerobic or anaerobic conditions and that PAB also exerts its anti-SA activity under anaerobic conditions. Growth and respiration are inhibited to somewhat the same extent as previously reported by Sevag[8] for *Streptococcus pyogenes* and Type I *Diplococcus pneumoniæ*. SA appears to inhibit respiration somewhere between the original dehydrogenase system and the final H-acceptor, possibly inhibiting a H-carrier system as well as the decarboxylase system. It is not evident whether the decreased rate of respiration is due to inhibition of growth or vice versa although Wyss[9] reports that respiration is inhibited by isomers of SA which do not exhibit "chemotherapeutic activity."

[8] Sevag, M. G., and Shelburne, M., *J. Bact.*, 1942, **43**, 447.

[9] Wyss, O., Strandskov, F. B., and Schmelkes, F. C., *Science*, 1942, **96**, 236.

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Alteration of Crystalline Androsterone and Dehydroisoandrosterone by Alcohol as a Vehicle.

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Investigations were undertaken to evaluate the respective merits of alcohol and oil as vehicles for androgenic assays after having observed[1] that the degree of response of the chick's comb to androsterone, dehydroisoandrosterone,* and varying mixtures of these compounds was modified by the vehicle in which they were dissolved. Klempner, Frank and Hollander[2] noted a greater increase in the weight of chicks' combs treated with crystalline androsterone dissolved in 95% alcohol over that resulting from the use of an oily solution. They consequently recommended alcohol as the vehicle of choice. The enhancement of tissue response was ascribed by them to either better absorption of the hormone from the alcoholic solution or to an increased rate of evaporation of the vehicle. Our results indicate that changes occur in such alcoholic solutions, making this vehicle unsatisfactory for biological assays of these androgens.

Experimental. In these investigations we employed the chick-comb-weight method, as previously described.[3] Although several workers[4,5,6] have questioned the value of the chick as a test animal, our extensive experience during the past 4 years, with routine and experimental androgenic assays, has shown

[1] Grauer, R. C., Starkey, W. F., and Saier, E., studies in progress.

* The androsterone and dehydroisoandrosterone used in these studies were kindly supplied by the Ciba Pharmaceutical Products through the courtesy of Dr. Ernst Oppenheimer.

[2] Klempner, E., Frank, R. T., and Hollander, F., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 633.

[3] Starkey, W. F., Grauer, R. C., and Saier, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 649.

[4] Duff, P. A., and Darby, H. H., *Endocrinology*, 1941, **28**, 643.

[5] McCullagh, D. R., and Guillet, R., *Endocrinology*, 1941, **28**, 648.

[6] Hoskins, W. H., Beach, G. W., Coffman, J. R., and Koch, F. C., *Endocrinology*, 1941, **28**, 651.