

not in the recumbent position. This change occurs at 10.5 seconds (average 3 determinations) in the sitting and at 10.2 seconds (average 13 determinations) in the standing position and is obviously due to recirculation⁸ although the factors involved in the more rapid recirculation in these positions have not yet been determined. These experiments account for the falsely high A-V O₂ differences and low outputs obtained with the Grollman procedure in the sitting and standing positions, since under these conditions the acetylene difference is erroneously low.

The adequacy of the short Gladstone rebreathing procedure for attaining homogeneity of gases in the lung-bag system prior to drawing the first sample has been substantiated. Two first samples were drawn simultaneously from the proximal and distal ends of the bag respectively. The resulting A-V O₂ differences calculated by pairing each of these first samples with the one second sample agreed closely. A-V O₂ differences calculated from any 2 samples in the 6-sample experiments also show good agreement from the end of the first expiration until recirculation takes place.

9197 P

Effect of Metabolites on Growth, and Differentiation in the Colon-Group.*

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The subdivision of the colon-group of bacteria into distinct subgroups is still a problem despite many studies. The genera *Escherichia* and *Aërobacter* can be differentiated from each other by nearly a dozen correlated characters. The genus *Aërobacter* can be further subdivided on correlated characters into at least 2 distinct subgroups represented by *Aërobacter aerogenes* and *Aërobacter cloacæ*. Aside from these divisions, however, considerable confusion still exists.

There are many strains whose characters are such that they cannot be allocated to either of the genera. In this paper these bacteria will be referred to collectively as "Intermediates".

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TABLE I.
Relative Vigor of Growth of Colon-aërogenes Strains on "Staled" Agar Medium.

Test Organism	Escherichia				"Intermediates"				<i>Aër. aerogenes</i>				<i>Aër. cloacæ</i>			
	a	b	c	d	e	f	g	h	i	1	m	n	o	r	s	t
Culture Substrata																
115	c	—	—	—	—	—	—	—	++	++	++	++	++	++	++	++
200	e	—	—	—	—	+	—	—	++	++	++	++	++	++	++	++
Escherichia "Staled" Agar.																
148	i	++	++	++	++	++	—	—	+	—	—	++	++	++	++	++
277	j	++	++	++	++	++	—	—	+	—	—	++	++	++	++	++
Intermediate-group "Staled" Agar.																
66A	1	++	++	++	++	++	++	++	++	++	++	++	++	—	++	++
244	o	+	++	++	++	++	++	++	++	++	++	++	++	—	++	++
<i>Aërobacter aerogenes</i> "Staled" Agar.																
252	s	++	++	++	++	++	++	++	++	++	++	++	++	—	—	++
279i	t	++	++	++	++	++	++	++	++	++	++	++	++	—	—	+
<i>Aërobacter cloacæ</i> "Staled" Agar.																

— No growth; + slight growth; ++ fair growth; +++ moderate growth.

Garré¹ and later Eijkman² showed that bacterial growth-products exhibit specific inhibitory effects. The purpose of this paper is to ascertain whether antagonism, or antibiosis, offers possibilities for differentiation of bacteria of the colon-group.

The method adopted was as follows: Broth containing 1.0% Bacto proteose-peptone and 0.1% K_2HPO_4 was inoculated with colon-organisms. These broth cultures were then incubated for 10 days at 37°C. An equal volume of a 3.0% agar gel was added to each of these 10-day broth cultures and plates were poured. This constituted what is known as a "staled" agar substratum.

These "staled" agar plates were then streaked with 24-hour broth cultures of the homologous organism and with 24-hour broth cultures of a number of other test-organisms employed in producing "staled" substrata. The cultures employed were 5 strains of the genus *Escherichia*, 6 "Intermediates", 6 *Aërobacter aërogenes*, and 6 *Aërobacter cloacæ*. Each of the 23 test-cultures was streaked on each of the 23 "staled" substrata, and the plates incubated for 48 hours at 37°C. Typical results are shown in Table I.

It is evident that the relative vigor of growth of an organism is distinctly better when streaked on a medium "staled" by an organism other than a member of its own genus or species than was the case with "staled" agar of the same genus or species. It is suggested that metabolic end-products (metabolites) are responsible for the effect observed.

Four 50 cc. samples of "staled" broth from 4 cultures were filtered through new, 155 mm., L5, Chamberland-Pasteur filter candles, agar gel added, and test cultures streaked as before. Distinct evidence of further growth was obtained, indicating that some of the inhibitory metabolites were adsorbed by the filters. Similarly, boiling a "staled" medium reduced its growth-inhibiting properties.

The results indicate the existence of adsorbable substances in cultures of *coli-aërogenes* organisms which seem to exhibit some evidence of specific, growth-inhibiting properties against homologous strains. It is suggested that these substances might assist in inter-group differentiation.

¹ Garré, C., *Centbl. Bakt.*, 1887, **2**, 312.

² Eijkman, C., *Centbl. Bakt., Abt. 1*, 1904, **37**, 436.