

Cyst Formation *in vitro* by *Entamoeba histolytica*. (24019)

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(Introduced by H. H. Anderson)

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In some laboratories, encystment *in vitro* of *Entamoeba histolytica* seems to occur spontaneously; our own experience has been that this phenomenon occurs but rarely. This difference may be due to the fact that our cultures have been mainly derived from cases of acute, ulcerative, amoebic colitis where the amoebae are, as a rule, large and active and in which cysts are very rarely found. Further, the numerous methods which have been described for inducing cyst formation in cultures by this amoeba have not proved successful by all observers and it is apparent that little is known of the factors underlying the process.

Materials and methods. Recently, by provision of suitable associates, we have been able to culture *E. histolytica* from cases of liver abscess (Freedman, Maddison and Elsdon-Dew(1)). The associates used include *Clostridium welchii*, *Escherichia coli* and a bacterial complex "M", derived from a previously successful stool culture of *E. histolytica*. Cysts were never seen in the lines cultured with either *Cl. welchii* or *Esch. coli* as associate, but with the "M" complex cysts have been found in small numbers quite regularly. This suggested that there was in the "M" complex some factor or factors which induced amoebae derived from liver to encyst. The "M" complex was therefore separated bacteriologically and the various species found, used singly and in combination, to discover which, if any, were of importance. The species found in the "M" complex are listed in Table I.

Two lines of *E. histolytica* called respectively "A" and "L" and both originally derived from human liver abscesses by provision of *Cl. welchii* as associate were used in the ex-

periments. In some tests the various organisms of the "M" complex were added to an *E. histolytica-Cl. welchii* culture; in others an *E. histolytica-Esch. coli* culture was used. In a few tests, *E. histolytica* freed of its primary associate was used. All tests were made in Locke-Egg tubes.

Results. Table II gives the results.

(i) *E. histolytica* "A" with *Cl. welchii* plus test organisms (Table II, column 1).

1. and 2. Confirm that cysts are found with the "M" complex and that by separation of constituent flora the encystment factor has not been lost.

3. Gives results for combinations of 5 of the original 6 organisms. Apparently omission of e or f does not affect cyst formation.

4. Shows that omission of both e and f does not affect encystment.

5. Indicates that in combinations of 3 of the 4 remaining organisms, c is apparently necessary.

6. Shows that in combinations of 2 organisms c is essential for encystment.

7. Gives results for the organisms one at a time. Encystment does not occur, even with c.

8. Shows that c in combination with e gives cysts but not in combination with f.

(ii) *E. histolytica* "A" with *Esch. coli* plus test organisms (Table II, column ii). This column indicates that for all combinations tested the presence of *Esch. coli* as the primary concomitant makes no difference to the results.

(iii) *E. histolytica* "A" plus test organisms (Table II, column iii). This column shows

TABLE I. Bacteria Comprising the "M" Complex.

M. a.	coliform group
M. b.	<i>Idem</i>
M. c.	gram negative fusiform bacillus. Anaerobe.
M. d.	gram positive bacillus. Aerobe.
M. e.	weakly gram positive bacillus. Aerobe.
M. f.	streptococcus

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TABLE II. Cyst Formation with Bacteria of the "M" Complex.

Bacterial combination with <i>E. histolytica</i>		"A" line			"L" line	
		With <i>Cl. welchii</i> (i)	With <i>E. coli</i> (ii)	No primary associate (iii)	With <i>Cl. welchii</i> (iv)	No primary associate (v)
1.	M. complex	+				
2.	M. a,b,c,d,e,f	+			+	
3.	M. a,b,c,d,e	+		—	+	+
	M. a,b,c,d, f	+		+	+	+
	M. a,b,c, e,f	—			+	+
	M. a,b, d,e,f	—			+	—
	M. a, c,d,e,f	—		—	+	—
	M. b,c,d,e,f	—(2)*		—	+	
4.	M. a,b,c,d	+(2)	+	—	+	+
5.	M. a,b, d	—				
	M. a,b,c	+	+	—(2)	+	—
	M. a, c,d	+	+		+(2)	—
	M. b,c,d	+	+	—(2)	+	—
6.	M. a,b	—				—
	M. a, c	+(2)	+		+	+
	M. b,c	+	+	—	+	+
	M. c,d	+	+	—	+	
	M. b, d	—				—
	M. a, d	—				
7.	M. a	—(2)		—		
	M. b	—(2)				
	M. c	—(2)		—		
	M. d	—(2)				
	M. e	—				
	M. f	—				
8.	M. e, e	+	+		+	
	M. e, f	—		—		—
	M. d,e				—	

+, cysts present; —, cysts absent.

* Except where stated one test only was done.

effect of removal of the primary concomitant by passage through the tissue-bearing medium of Shaffer, Sienkiewicz and Washington(2). Here the results were disappointing in that the only success was attained with the combination a,b,c,d,f.

(iv) *E. histolytica* "L" with *Cl. welchii* plus the test organisms (Table II, column iv). With "L" amoebae and *Cl. welchii* as primary associate the results were similar to those with "A" but encystment occurred here with all combinations of 5 organisms.

(v) *E. histolytica* "L" plus the test organisms (Table II, column v). With "L" amoebae and no primary associate, the results differed from those with *Cl. welchii* in that no cysts were found in several instances (3 and 5).

Conclusions. 1) A search for the encystment promoter amongst the organisms comprising a bacterial complex "M" which fosters cyst for-

mation *in vitro* of *E. histolytica* has been made. 2) From study of one line of amoebae "A" it appeared that organism c was essential, but that no one organism (including c) could by itself promote cyst production. Some balance or combination of products or conditions appears to be the requirement. There is a suggestion that one organism (f) may in some way be antagonistic to cyst formation. 3) Another line of amoebae "L" gave similar results to "A" but certain additional combinations of 5 organisms gave cysts. This may indicate a strain difference but, because of the small numbers of cysts found in all tests, we feel that the observation requires verification.

1. Freedman, L., Maddison, S. E., Elsdon-Dew, R., *S.A.J. Med. Sci.*, 1958.

2. Shaffer, J. G., Sienkiewicz, H. S., Washington, J. E., *Am. J. Hyg.*, 1953, v57, 366.

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