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Research Note

Osmotic Pressure-Dependent Development of Microfilariae of *Dirofilaria immitis in Vitro*

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Various kinds of media have been used for the cultivation of microfilariae (mf) of filarial worms *in vitro*. Among them, NCTC-109, NCTC-135 and Grace's medium, when supplemented with various kinds of sera, supported the development of mf to the "sausage stage" (Sawyer and Weinstein, 1963; Devaney and Howells, 1979; Klein and Bradly, 1974), whereas neither MEM nor 199 medium did (Earl, 1959; Ando *et al.*, 1980 b). The osmotic pressure of the three media which supported the development of mf was higher than that of the two which did not support development, so the relationship between osmotic pressure of culture media and development of *D. immitis* was studied *in vitro*.

The osmotic pressure of several media and dog plasma was measured with an osmometer (Vogel, Western Germany) and the values are shown in Table 1. Distilled water and/or 20% to 40% sucrose solution were added to the culture media to make the required osmotic pressure (±2 mOsm/Kg); i.e. 80 part of medium, 10 part of fetal bovine serum (FBS), 10 part of distilled water and/or 20% to 40% sucrose solution. Mf in a naturally infected dog were isolated by the precedure described by Ando *et al.* (1980 a). The mf were washed three times in the culture medium in which they were to be tested. One ml of culture medium containing approximately 200 mf was incorporated into a bottle measuring 15×15×150 mm. Bottles with rubber stopper were then kept at 27 C without renewing the medium throughout the experiment. The first stage larvae were classified according to the method of Sawyer and Weinstein (1963).

The number of type IV increased with the elevation of the osmotic pressure in the NCTC-109 medium supplemented with 10% FBS (Table 2). The favorable osmotic pressure ranged from 330 mOsm/Kg to 390 mOsm/Kg. It was interesting evidence that at 420 mOsm/Kg there was development of early forms to some extent but no development to type IV. Generally under the various culture conditions "developing forms" survived over 6 days, while the undeveloped mf died earlier. The number of type IV did not increase significantly in either F-12 or RPMI-1640 media supplemented with 10% FBS in which the osmotic pressure was not adjusted. However the number of type IV increased apparently in the same media adjusted at 330 mOsm/Kg. Mf never developed in MEM, 199 media both with 10% FBS and NCTC-109 without FBS at 330 mOsm/Kg. Therefore we have to consider factor(s) included in the medium and FBS which might be necessary for the development of mf.

Although the osmotic pressure of dog plasma usually ranged from 285 to 305 mOsm/Kg (Bovee, 1969), it was slightly

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Table 1 The osmotic pressure of various culture media and other solutions

Medium	Osmotic pressure (mOsm/Kg)*		Remarks
	without FBS	with FBS (10% final conc.)	
199	275.7±0.6	277.7±0.6	Powder, Nissui Seiyaku
MEM	269.7±0.6	273.0±1.4	Powder, Nissui Seiyaku
RPMI-1640	267.0±1.0	270.0±1.0	M.B.A. Maryland
F-12	294.3±1.0	294.3±1.3	M.B.A. Maryland
NCTC-109	294.3±0.6	294.3±0.6	M.B.A. Maryland
NCTC-135	295.7±0.6	295.3±0.6	M.B.A. Maryland
Grace	353.6±1.5	348.5±1.3	Gibco N.Y.
Dog plasma	310.0±0.6		
FBS	310.3±0.6		Gibco N.Y.

*: Values in the table indicate mean ± standard deviation (three to five measurements)

Table 2 The osmotic pressure-dependent development of microfilariae in each medium after 16 days culture

Medium*	Osmotic pressure (mOsm/Kg)	Development (%)†			
		Type I (mf-type)	Type II (intermediate)	Type III (early sausage)	Type IV (sausage)
NCTC-109	270	77.6± 6.4	18.6± 5.5	3.0±1.6	0.8± 0.5
	285	62.9±12.4	18.6±10.0	12.8±2.7	5.7± 2.6
	300	28.4± 8.0	20.3± 6.7	27.3±6.4	24.0± 7.2
	315	15.4± 6.6	23.4±11.1	26.8±5.6	34.4± 8.6
	330	7.0± 3.8	9.7± 7.5	28.7±5.9	54.6±12.8
	345	8.2± 3.9	9.5± 5.0	33.6±6.9	48.8± 7.5
	360	6.2± 4.3	9.4± 2.9	28.8±4.0	55.7± 8.4
	390	5.2± 3.5	5.7± 3.7	33.8±4.9	55.3± 8.4
	420	83.7± 8.2	14.5± 7.2	1.8±1.2	0
F-12	294	85.8± 5.8	7.0± 1.3	3.4±2.4	3.7± 2.3
	330	46.2± 9.8	18.1± 2.9	16.1±2.1	19.7± 7.8
RPMI-1640	270	97.4± 2.0	1.6± 1.7	0.6±0.4	0.4± 0.5
	300	18.3± 8.2	12.9± 4.7	21.7±3.4	47.2± 9.7

*: Each medium was supplemented with 10% FBS (final conc.).
†: Values in the table indicate mean ± standard deviation (eight bottles).

heigher, 310 mOsm/Kg, in two laboratory bred dogs (one infected and the other non-infected). The favorable osmotic pressure of culture media for the development of mf was higher than that of natural dog plasma in which mf live. Therefore, it would be speculated that the development of mf to the "sausage stage" is due to the increase of osmotic pressure in the mid gut of the mosquito when the dog plasma reaches there.

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In vitro における *D. immitis* のミクロフィラリアの浸透圧に依存した発育

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D. immitis のミクロフィラリアを浸透圧の異なる NCTC-109 培地で培養した結果，浸透圧の上昇につれて第一期後期幼虫に発育する割合は増加し，至適浸透圧は 330～390 mOsm/kg の範囲であった．F-12 及び RPMI-1640 培地においても浸透圧を 330 mOsm/kg に調節すれば第一期後期幼虫にまで発育する割合はいちじるしく増加したが 199 及び MEM 培地では浸透圧を調節しても第一期後期幼虫にまで発育しなかった（培地はいずれも 10% FBS 添加）．