Production of Single-walled Cysts of *Schizopyrenus russelli* by Sonication and their Behaviour Towards Excystment-inducing and Excystment-blocking Agents

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SUMMARY

Ultrasonic treatment of double-walled cysts of *Schizopyrenus russelli* led to the removal of the outer wall. These single-walled cysts excysted readily in aqueous extract of *Escherichia coli*, as did normal double-walled cysts. There was a marked delay in the excystment of single-walled cysts obtained by ultrasonic treatment of the normal cysts treated with *p*-chloromercuric benzoate (*p*-CMB) or emetine, similar to that obtained with normal cysts. The action of *p*-CMB was annulled by reduced glutathione. These findings suggest that the sites of action of excystment-inducing and excystment-blocking agents are on or inside the inner cyst wall and that these agents are able to permeate the outer cyst wall.

INTRODUCTION

Earlier work has shown that aqueous extracts of either *Aerobacter* species or *Escherichia coli*, or certain amino acids, are able to cause excystment of double-walled cysts of the amoeba *Schizopyrenus russelli* Singh (Singh, Mathew & Anand, 1958). Emetine inhibited the induction of excystment by these agents (Imam, Dutta & Agarwala, 1968) and *p*-chloromercuric benzoate (*p*-CMB) delayed the emergence of trophozoites from the cysts (Rastogi, Sagar & Agarwala, 1969). This action of *p*-CMB was annulled by reduced glutathione. The present paper deals with the production of single-walled cysts of *S. russelli* by ultrasonic treatment and their behaviour towards excystment-inducing and excystment-blocking agents.

METHODS

Schizopyrenus russelli was grown in monobacterial culture with *Escherichia coli*. Viable sterile cysts, free from bacteria, were obtained by the method reported earlier (Rastogi *et al.* 1969). A suspension of 10×10^6 cysts/ml. was made and stored in a refrigerator. Ultrasonic treatment was done for periods up to 10 min. in 8 ml. lots of cyst suspension in distilled water containing 2×10^7 cysts in a 50 ml. beaker, chilled in crushed ice, by using a Mullard ultrasonic generator fitted with 20 kc. transducer (output current 1.5 amperes). The treated cysts were centrifuged at 650 g for 5 min. and suspended in distilled water to 10×10^6 cysts/ml.; 0.1 ml. of this suspension was added to 0.9 ml. aqueous extract of *E. coli* and the excystment followed as reported earlier (Rastogi *et al.* 1969).

RESULTS

Table 1 shows that ultrasonic treatment of double-walled cysts (Pl. 1, fig. 1) removed the outer wall and increased the number of single-walled cysts (Pl. 1, fig. 2). A decrease in the total count (normal and single-walled cysts) was also observed, indicating cyst disintegration. After 2 min., 59% of the cysts were converted to single-walled structures, and 29% of the cysts were completely disrupted. After more than 3 min. treatment, the residual population comprised almost entirely single-walled cysts. The single-walled cysts obtained by ultrasonic treatment excysted readily in *Escherichia coli*

Table 1. *Effect of ultrasonic treatment of cysts of Schizopyrenus russelli*

Exposure time (min.)	% of cysts		
	Double-walled	Single-walled	Disrupted completely
0	100	0	0
$\frac{1}{2}$	75	17	8
1	30	47	23
2	12	59	29
3	2	53	45
5	1	25	74
10	0	5	95

Table 2. *Effect of p-chloromercuric benzoate on excystment of ultrasonically treated single-walled cysts of Schizopyrenus russelli*

Cysts treated with	Hr... Stage...	% count of different stages after varying periods of treatment															
		2				4				8				20			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
<i>E. coli</i> extract (normal cysts)		75	0	5	20	40	0	7	53	10	0	20	70	2	0	2	96
<i>E. coli</i> extract (ultrasonically treated cysts)		12	50	4	34	0	45	0	55	0	35	0	65	0	5	0	95
Ultrasonically treated cysts treated with <i>p</i> -CMB (10^{-4} M) in <i>E. coli</i> extract		10	80	6	4	10	78	4	8	4	76	0	20	0	50	0	50
Ultrasonically treated cysts treated with <i>p</i> -CMB (10^{-4} M) for 1 hr; excystment with <i>E. coli</i> extract after the removal of <i>p</i> -CMB		10	89	0	1	8	72	5	15	4	69	6	21	0	50	0	50
Ultrasonically treated cysts treated with <i>p</i> -CMB (10^{-3} M) in <i>E. coli</i> extract		12	88	0	0	12	88	0	0	12	88	0	0	12	88	0	0
Ultrasonically treated cysts treated with <i>p</i> -CMB (10^{-3} M) for 1 hr; excystment in <i>E. coli</i> extract after the removal of <i>p</i> -CMB		14	86	0	0	8	86	6	0	7	85	8	0	6	83	11	0

A, Double-walled cysts; B, single-walled cysts; C, cysts in the process of excystment; D, trophozoites. Ultrasonic treatment time 3 min.

extract, as did normal double-walled cysts (Tables, 2, 4 and 6). The majority of them did not take eosin staining (0.125%, w/v), suggesting that the cysts were viable.

Effect of p-CMB on excystment of single-walled cysts

The excystment of single-walled cysts of *Schizopyrenus russelli* was similar to that of the double-walled cysts (Table 2). When the single-walled cysts were treated with *p*-CMB (10^{-4} M) in *Escherichia coli* extract, there was a marked delay in excystment. Similar results were obtained when the cysts were treated with *p*-CMB (10^{-4} M) in water, washed in distilled water and then put for excystment in *E. coli* extract. Under both these conditions cysts treated with 10^{-3} M *p*-CMB failed to excyst in 20 hr. Reduced glutathione (GSH) annulled the *p*-CMB effect, both when added with *p*-CMB and after removal of the latter (Table 3). There was a marked delay in excystment of the single-walled cysts of *S. russelli* obtained by sonication of the normal cysts treated with *p*-CMB (Table 4). These findings suggest that *p*-CMB is able to permeate the outer cyst wall of double-walled cysts.

Table 3. *Annulment of p-CMB delayed excystment of ultrasonically treated Schizopyrenus russelli cysts by reduced glutathione*

	% count of different stages after varying periods of treatment																
	Hr...	2				4				10				20			
Cysts treated with	Stage...	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
<i>E. coli</i> extract (normal cysts)		56	0	11	33	45	0	0	55	8	0	2	90	5	0	0	95
<i>E. coli</i> extract (ultrasonically treated cysts)		7	49	5	39	0	37	0	63	0	6	0	94	0	6	0	94
Ultrasonically treated cysts treated with <i>p</i> -CMB (10^{-3} M) for 1 hr; excystment in <i>E. coli</i> extract after the removal of <i>p</i> -CMB		11	89	0	0	12	88	0	0	12	88	0	0	12	88	0	0
Ultrasonically treated cysts treated with <i>p</i> -CMB (10^{-3} M) + GSH (2×10^{-3} M) for 1 hr; excystment in <i>E. coli</i> extract after the removal of <i>p</i> -CMB and GSH		7	55	4	34	0	41	0	59	0	9	0	91	0	10	0	90
Ultrasonically treated cysts treated with <i>p</i> -CMB (10^{-3} M) for 1 hr; washed and then treated with GSH (2×10^{-3} M) for 1 hr; excystment in <i>E. coli</i> extract after the removal of GSH		12	88	0	0	0	56	0	44	0	40	0	60	0	30	0	70

A, B, C and D as in Table 1. Ultrasonic treatment for 3 min.

Effect of emetine on excystment of normal and ultrasonically treated cysts

The results in Table 5 indicate that, even after the removal of emetine, there was a delay in excystment which was proportional to the concentration of emetine. Ultrasonically treated single-walled cysts were also sensitive to the action of emetine (Table 6) and the delay in excystment increased with its concentration. The maximum

excystment obtained in 24 hr after incubation and removal of emetine (10 mg./ml.) was around 50% (Tables 4 and 5). The residual cysts, however, excysted almost completely in another 24 hr when incubated with fresh bacterial extract.

When emetine-treated normal cysts were subsequently treated ultrasonically, a

Table 4. *Delay in excystment of single-walled cysts of Schizopyrenus russelli (obtained by ultrasonic treatment of normal cysts treated with p-CMB or emetine)*

Cysts treated with	Hr... Stage...	% count of different stages after varying periods of treatment															
		2				4				8				20			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
<i>E. coli</i> extract (normal cysts)		58	0	4	38	53	0	0	47	15	0	0	85	5	0	0	95
<i>E. coli</i> extract (ultrasonically treated cysts)		0	80	0	20	0	62	0	38	0	30	0	70	0	8	0	92
Normal cysts treated with <i>p</i> -CMB (10^{-3} M) for 1 hr; excystment with <i>E. coli</i> extract after the removal of <i>p</i> -CMB		100	0	0	0	100	0	0	0	79	0	21	0	70	0	20	10
Normal cysts treated with <i>p</i> -CMB (10^{-3} M) for 1 hr, and washed; excystment with <i>E. coli</i> extract after ultrasonic treatment		0	100	0	0	0	100	0	0	0	75	0	25	0	70	0	30
Normal cysts treated with emetine (10 mg./ml.) for 1 hr; excystment in <i>E. coli</i> extract after removal of emetine		100	0	0	0	100	0	0	0	100	0	0	0	50	0	0	50
Normal cysts treated with emetine (10 mg./ml.) for 1 hr, and washed; excystment with <i>E. coli</i> extract after ultrasonic treatment		0	100	0	0	0	100	0	0	0	100	0	0	0	55	0	45

A, B, C, and D as in Table 1.

Table 5. *Delay in excystment of normal cysts of Schizopyrenus russelli treated with emetine*

Cysts treated with	Hr... Stage...	% count of different stages after varying periods of treatment								
		4			8			24		
		A	B	C	A	B	C	A	B	C
<i>E. coli</i> extract (normal cysts)		20	33	47	16	8	76	0	0	100
Emetine (0.1 mg./ml.) for 1 hr; excystment in <i>E. coli</i> extract after removal of emetine		17	36	47	13	22	65	0	0	100
Emetine (1 mg./ml.) for 1 hr; excystment in <i>E. coli</i> extract after the removal of emetine		34	32	34	10	32	58	0	0	100
Emetine (10 mg./ml.) for 1 hr; excystment in <i>E. coli</i> extract after the removal of emetine		100	0	0	91	0	9	52	0	48

A, Double-walled cysts; B, cysts in the process of excystment; C, trophozoites.

delay in excystment was still observed in the resulting single-walled cysts, as found with *p*-CMB (Table 4), showing that emetine also was able to permeate the outer cyst wall of normal double-walled cysts.

Table 6. *Delay in excystment of ultrasonically treated cysts of Schizopyrenus russelli treated with emetine*

		% count of different stages after varying periods of treatment															
Hr...		2				4				8				20			
Cysts treated with	Stage...	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
<i>E. coli</i> extract (normal cysts)		10	0	8	82	5	0	0	95	1	0	0	99	1	0	0	99
<i>E. coli</i> extract (ultrasonically treated cysts)		0	70	0	30	0	39	0	61	0	26	0	74	0	4	0	96
Ultrasonically treated cysts treated with emetine (0.1 mg./ml.) for 1 hr; excystment in <i>E. coli</i> extract after the removal of emetine		0	100	0	0	0	90	0	10	0	36	0	64	0	10	0	90
Ultrasonically treated cysts treated with emetine (1 mg./ml.) for 1 hr; excystment in <i>E. coli</i> extract after the removal of emetine		0	100	0	0	0	100	0	0	0	84	0	16	0	70	0	30
Ultrasonically treated cysts treated with emetine (10 mg./ml.) for 1 hr; excystment in <i>E. coli</i> extract after the removal of emetine		0	100	0	0	0	100	0	0	0	96	0	4	0	85	0	15

A, B, C and D as in Table 1. Ultrasonic treatment for 3 min.

DISCUSSION

Ultrasonic treatment of *Schizopyrenus russelli* cysts gave single-walled structures. These single-walled cysts excysted in *Escherichia coli* extract as readily as the normal double-walled cysts. This suggests that the site of interaction with the excystment factors is located either on or within the inner cyst wall and that the excystment agents are able to permeate the outer cyst wall to trigger the excystment mechanism in normal cysts. The marked delay in excystment of single-walled cysts after treatment with *p*-CMB or emetine was similar to that obtained with normal cysts, and suggests that these agents are able to permeate the outer cyst wall. The delay in excystment depended on the concentration of *p*-CMB and emetine used.

The fact that *p*-CMB- and emetine-treated cysts were viable and could excyst after longer incubation in the bacterial extract shows that the damage caused by these agents is repairable by factors inherent within the cysts or in the excystment medium.

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EXPLANATION OF PLATE

Fig. 1. Normal double-walled cysts of *Schizopyrenus russelli*.

Fig. 2. Ultrasonically treated single-walled cysts of *S. russelli* after the removal of the outer wall.

Fig. 1

Fig. 2