NOTE

**Effect of Bile Salts on *in vitro* Excystation
of *Eimeria tenella* Oocysts**

Toshimitsu TOYAMA, and Noritoshi KITANO

Sankyo Co., Ltd., Biological Research Laboratories, 1-2-58 Hiromachi, Shinagawa, Tokyo 140

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Doran and Farr [3] and Farr and Doran [4] reported that the oocyst wall of poultry coccidia was crushed mechanically in the gizzard and the sporozoites were liberated in the small intestine. Moreover, *in vitro* excystation of poultry coccidia occurred when sporocysts were treated with trypsin and bile. In subsequent studies, the necessity of trypsin and bile in excystation of oocysts of *Eimeria* has been substantiated [1, 2, 5]. In this investigation, the authors also recognized the significance of trypsin and bile for excystation and confirmed the usefulness of commercial bile salts like the chicken bile on the *in vitro* excystation.

Oocysts were obtained from the caeca of chickens 7 days after infection with *Eimeria tenella*. Sporulation was performed by incubating the oocysts in a 2% potassium dichromate solution for more than 3 days at 25°C. The sporulated oocysts disinfected in a 5–6% sodium hypochlorite solution and were stored in sterilized 0.1 M phosphate buffered saline, pH 7.2, (PBS) at 5°C. The sporocysts were prepared by crushing the oocyst wall with a Dounce-type homogenizer and incubated in the excysting solution at 37°C or 41°C for 1 h. They were placed in an ice bath immediately after incubation. Excystation rates were

calculated as follows:

$$\text{Rate of empty sporocysts} = \frac{\text{Number of empty sporocysts}}{\text{Number of all sporocysts}} \times 100$$

$$\text{Rate of free sporozoites} = \frac{\text{Number of free sporozoites}}{\text{Number of empty sporocysts} \times 2} \times 100$$

The excysting solution was composed of trypsin (1:250, Difco) and pooled chicken bile or each of bile salts in PBS. The bile salts were as follows: cholic acid sodium salt (C, Sigma), taurocholic acid sodium salt (TC, Nakarai Chemical Ltd.), glycocholic acid sodium salt (GC, Sigma), deoxycholic acid sodium salt (DC, Nakarai Chemical Ltd.), taurodeoxycholic acid sodium salt (TDC, Sigma), glycodeoxycholic acid sodium salt (GDC, Sigma), chenodeoxycholic acid (CDC, Sigma), lithocholic acid sodium salt (LC, Calbiochem-Behring Corp.), and taurochenodeoxycholic acid sodium salt (TCDC, Sigma). The concentrations of these materials in the excysting solution are indicated in Table 1.

As shown in Table 1, chicken bile was the best excystor at 41°C. When sporocysts were incubated in C and in GC, the rates of empty sporocysts showed such low levels as lower than 71.4% and 73.1%, respectively, but the rate of free sporozoites attained such high levels as

Table 1. Effect of bile salts in an excysting solution on excysting

Concentration of		Excysting solution consisting									
Bile salt(%)	Trypsin(%)	C(41°C)		TC(41°C)		GC(41°C)		DC(41°C)		TDC(41°C)	
		ES	FS	ES	FS	ES	FS	ES	FS	ES	FS
0.05	0.25	14.2	84.8	28.7	48.1	15.6	87.7	59.3	6.3	53.4	92.3
0.1	0.25	35.7	94.6	53.5	76.9	19.4	88.7	88.6	0	86.4	95.8
0.2	0.25	64.8	83.4	74.1	100	45.6	97.7	21.4	0	92.2	65.5
0.3	0.25	71.4	82.2	88.1	93.5	66.7	92.7	6.3	0	90.3	51.2
0.4	0.25	63.2	85.5	88.7	99.4	73.1	72.3	3.3	0	94.6	64.1
0.5	0.25	70.5	64.5	93.1	76.4	50.9	82.5	2.3	0	95.8	51.3
—	0.25	18.6	68.5	19.0	86.2	13.9	78.2	14.1	36.3	15.0	86.7
0.5	—	0.4	0	2.8	0	0	0	2.3	0	1.4	0
Control (Pooled chicken bile 10% + Trypsin 0.25%)		99.3	92.0	97.2	100	98.4	95.0	97.4	89.4	98.0	90.1

C: Cholic acid sodium salt, TC: Taurocholic acid sodium salt, GC: Glycocholic acid sodium salt, DC: sodium salt, CDC: Chenodeoxycholic acid, LC: Lithocholic acid sodium salt, TCDC: Taurochenode-crystal structures were observed.

94.6% and 97.7%, respectively. With increasing the concentration of TDC and TCDC, a tendency of decrease in the rate of free sporozoites was observed. These bile salts seemed to be less effective excystors for *E. tenella*. GDC, one of the bile salts, appeared to be the best excystor among all bile salts tested, being as good as chicken bile. The concentration giving the best result was 0.3% to 0.5%, and the rates of empty sporocysts and free sporozoites were 97.1% and 93.6%, respectively. TC seemed to follow GDC. On the other hand, lysis of free sporozoites in the excysting solution with CDC and degeneration of sporocysts in the excysting solution with DC or LC were observed.

When trypsin was used alone without chicken bile or any bile salt as an excysting stimulus, only a small number of sporozoites of *E. tenella* excysted, and no sporozoite excysted with chicken bile or bile salts alone.

When incubated at 37°C, the rate of

empty sporocysts decreased obviously in C but did not change in GDC at a concentration of 0.2% or higher.

From the results of these experiments, in which no relation was found on the activity between taurin and glycin conjugates, it was assumed that chicken bile and bile salts helped trypsin in digesting the surface protein of the sporocysts.

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solution on excystation of sporozoites of *E. tenella*.

of trypsin and

GDC(41°)		CDC*(41°C)		LC*(41°C)		TCDC(41°C)		C(37°C)		GDC(37°C)	
ES	FS	ES	FS	ES	FS	ES	FS	ES	FS	ES	FS
28.2	85.3	72.9	0	5.8	0	71.8	78.3	2.7	54.5	3.2	35.0
76.9	77.8	87.2	0	0	0	93.0	85.5	3.4	46.2	10.9	77.6
75.2	90.5	92.2	0	0	0	89.0	94.3	2.8	31.8	79.1	91.3
95.2	93.6	96.5	0	2.5	0	93.2	71.7	4.5	43.8	90.6	88.9
97.1	93.6	100	0	0	0	94.8	58.8	5.9	58.3	85.3	100
95.3	91.1	97.9	0	3.6	0	93.0	55.4	9.5	48.6	82.0	100
15.3	96.2	36.5	89.3	23.2	83.6	12.2	82.8	1.6	33.3	0.9	75.0
1.7	0	3.4	0	2.4	0	1.0	0	0	0	0	0
97.5	93.8	98.7	87.9	99.4	87.5	96.0	92.8	62.8	91.5	61.2	88.1

Deoxycholic acid sodium salt, TDC: Taurodeoxycholic acid sodium salt, GDC: Glycodeoxycholic acid sodium salt, ES: Rate of empty sporocysts (%), FS: Rate of free sporozoites (%), * Some

要 約

Eimeria tenella の脱シストにおける胆汁酸塩の影響(短報): 遠山俊光・北野訓敏(三共株式会社生物研究所)——種々の胆汁酸塩について *Eimeria tenella* の人工脱シスト試験を行なった。41°C の感作温度ではトリプシン-ニワトリ胆汁からなる人工消化液が最もすぐれた成績を示した。胆汁酸塩のなかではグリコデオキシコール酸ナトリウムが胆汁に匹適し、タウロコール酸ナトリウムがこれにつぐ成績を示した。いっぽう、スポロシストやスポロゾイトの変性をもたらす胆汁酸塩も存在した。37°C 感作でもグリコデオキシコール酸ナトリウムの効果に影響は認められなかった。