Lysis of *Diphyllobothrium erinacei* plerocercoid by bile salts *in vitro*

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Introduction

The previous work (Furukawa, 1968) indicated that bile is one of the indispensable factors for the normal growth and survival of *Diphyllobothrium erinacei** in the intestine of the dog which is one of the final hosts of this species. At the same time, it was suggested that factors which stimulate the first step of growth, other than bile, may exist. To ascertain this point, it seems desirable to examine the direct action of bile on the early stage of growth, *i. e.* plerocercoid.

Recently, it has become clear that the chemical composition of bile, especially the nature of bile salts in bile, varies considerably from species to species (Haslewood, 1964), and that respective bile salts exert different effects on various stages in the life cycle of parasites (Smyth & Haslewood, 1963). It is likely to suppose that the major part of the effects of bile on the intestinal parasites and its peculiality may be attributable to the action of characteristic bile salts in bile.

Accordingly, the present investigation was performed to know the direct action of several bile salts on the plerocercoid of *D. erinacei in vitro*.

Materials and Methods

Plerocercoids of *D. erinacei* obtained from snakes or mice were sterilized through repeated washing with sterile physiological saline containing 2000 unit per ml of penicillin G (Takahashi *et al.*, 1959). In order to standardize the materials, the anterior part of the worm, 10 mm in length, was used.

Each of the bile salts was dissolved in Earle's solution at pH 7.2–7.4, and then sterilized through Seitz filter. The concentrations of sodium deoxycholate (M/1000), sodium cholate and sodium taurocholate (M/250 and M/100) were chosen empirically to be equivalent to approximately 10 and 25 per cent dog bile, and the concentration of sodium glycocholate (M/500 and M/250) was equivalent to 5 and 10 per cent human bile (Smyth, 1962).

Ten or 15 of sterilized plerocercoids were placed in 20 ml of the bile salt solution in a 50 ml Erlenmeyer flask with a silicone rubber stopper, and were incubated at 37°C. Gas phase was 5 % CO_2 in nitrogen (Berntzen & Mueller, 1964).

Worms in culture were examined for gross transformation and activity with an aid of a dissecting microscope at various intervals, and were classified into one of the following categories: (a) worms which were partially disintegrated and were active, (b) worms in which almost all of their body region were collapsed, but still alive, and (c) dead worms.

Results

Survival of plerocercoids in control medium (Earle's solution) after 8 days culture was 100 per cent.

The extent of effects of respective bile salts on the plerocercoid during 5 days culture are indicated in Figure 1.

Sodium deoxycholate Plerocercoids were rapidly lysed in M/1000 sodium deoxycholate solution and almost all of worms were dead within 24 hours. Typical pictures of this

* Synonym, *D. mansoni* (Iwata, 1968)

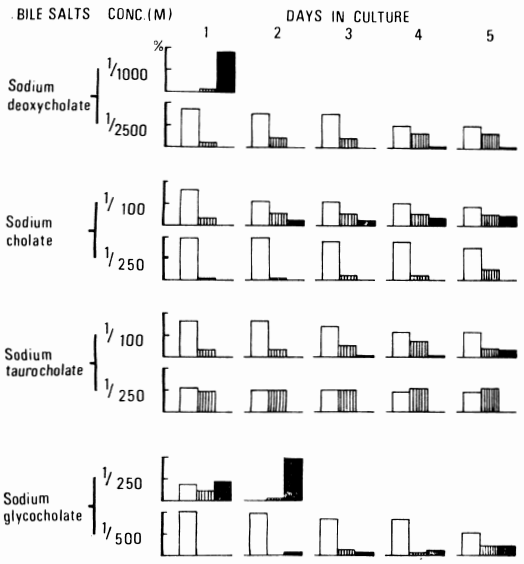

Figure 1. Effects of several bile salts on the plerocercoid of *D. erinacei in vitro*. Each of the columns represents the percentage of classified worm counts among total inoculum of 45 to 60 larvae.

- Partially disintegrated plerocercoids in normal activity.
- Worms in which almost all of the body region was collapsed.
- Dead worms.

process are indicated in Photos 1, 2 and 3. After several minutes of introduction into the medium, the plerocercoid body was cut to pieces and disintegrated (Photos 1, 2. Category a). Almost all of the body region of the worm was thus collapsed within several hours and eventually only scolex was remained (Photo. 3. Category b). Leaving scoleces did not undergo any further transformation, soon ceased the peristartic movement and then died (Category c). In dilution of less than M/2500, this salt had only a slight lytic effect.

Sodium cholate At M/100 concentration, sodium cholate killed one quarter of worms for 5 days in the same manner described above. Except for the partial disintegration of the body region, almost all of the worms were maintained in vigorous condition for 5 days in dilution of less than M/250.

Sodium taurocholate The result obtained with sodium taurocholate was similar to those with sodium cholate.

Sodium glycocholate This salt proved somewhat toxic lysing and killing all plerocercoids within 2 days at M/250 concentration by the same process as that observed in sodium deoxycholate. In dilution of less than M/500, this salt had a little observable effect during 5 days culture.

Discussion

It has been recognized that the characteristic bile salts are responsible for the initial establishment and subsequent growth of several larval cestodes (Berntzen & Mueller, 1964; Edgar, 1941; Rothman, 1959; Taylor, 1963; Yoshino, 1933). In the present work, it was demonstrated that four kinds of bile salts examined had a lytic action on the plerocercoid body, but the scolex region was not subjected to this effect although the prolonged culture resulted in the death of remaining scoleces. The evagination of scolex was observed occasionally in leaving worms, which were morphologically identical to the young worms recovered from the intestine of dogs approximately 6 hours after oral administration of plerocercoids (Photo. 4). In these respects, the transformation of plerocercoids in bile salt solutions appears to have a good agreement with that in the intestine of dogs when plerocercoids are taken by them (Iwata, 1933; Takahashi, 1959 a; Mueller, 1965). However, no evidence was obtained with the stimulative effect of bile salts on the development of the worm. This fact may partly supports the previous observation which indicated that plerocercoids can survive and grow for the first 3 days of infection even in the bile duct-ligated dog (Furukawa, 1968).

A remark shoud be given to the fact that sodium salts of deoxycholic and glycocholic acids had a marked effect on the plerocercoid rapidly lysing and killing these worms at relatively low concentration. In contract, cholic acid and its taurine conjugate seemed to have a little effect. It is probable that bile containing deoxycholic acid or glycine conjugated bile acids over a certain threshold

concentration may exert some deleterious effects on the establishment and growth of plerocercoids in the intestine of the host. From this point of view, it is noteworthy that the bile in suitable definitive host of the worm, such as dogs or cats, is consisted mainly with cholic acid flequently conjugated with taurine (Kazuno *et al.*, 1951; Mori, 1938; Tamura & Oono, 1953). These findings add support to the view that specific bile salts may act as selective biochemical agents in host specificity by eliminating parasites from the intestine of unsuitable hosts and by giving some stimulative effects in suitable ones (De Waele, 1934; Smyth, 1962; Smyth & Haslewood, 1963).

Smyth (1962) suggested that the composition of bile in animals which act as definitive hosts for parasites should have some common biochemical pattern, and the same probability may also be true of the composition of bile in intermediate hests. This, however, does not apply in the case of the intermediate host of *D. erinacei* since it was evidenced that bile in several intermediate hosts of this worm, such as primates including man, rabbits and hamsters, contains a great amount of deoxycholic and glycine conjugated bile acids (Blomstrand, 1961; Haslewood, 1964; Plange *et al.*, 1962; Wiggins & Wootton, 1958), while that in several other intermediate hosts, such as hedgehogs, rats, mice and snakes, has cholic acid and its taurine conjugates as main components (Haslewood & Wootton, 1950). It is conceivable, therefore, that as far as *D. erinacei* is concerned the characteristic bile salts in bile play some, but not always definitive, role in determining host specificity of this species.

Summary

Plerocercoids of *Diphyllobothrium erinacei* were incubated in four kinds of bile salt solutions made up in Earle's solution at pH 7.2–7.4, under a gas phase of 5% CO_2 in nitrogen at 37°C.

It was demonstrated that bile salts examined had a lytic action on the plercercoid body and often killed worms in a characteristic manner. No stimulative effects on the development of plerocercoids was observed in any of the bile salt solutions.

Of the bile salts examined, solium deoxycholate and sodium glycocholate, in dilu. tion of M/1000 and M/250 respectively, are found to exert a marked effect lysing and killing plerocercoids within a short period. On the contrary, sodium cholate and sodium taurocholate, at less than M/100 concentration, had a little observable effect.

The chemical nature of bile and its role in parasitism of *D. erinacei* was discussed with several definitive and intermediate hosts of this worm.

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Explanation of Photographs

The disintegration process of *D. erinacei* plerocercoids in M/1000 sodium deoxycholate. Materials were fixed by 10 % formalin and stained with Meyer's borax carmine.

Photo. 1. Cutting off of the pleroceroid body. ×25.

Photo. 2. Lysis of the body at the posterior extremity. Note the invaginating scolex. × 60.

Photo. 3. Evaginated scolex. Almost all of the body region was collapsed. Note the morphological resemblance to the worm indicated in Photo. 4. ×120.

Photo. 4. A juvenil worm recovered from the jejunum of the dog approximately 6 hours after oral administration of plerocercoids. ×120.

マンソン裂頭条虫 Plerocercoid におよぼす胆汁酸の影響

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前報で，総胆管を結紮したイヌに，マンソン裂頭条虫の Plerocercoid を経口投与すると，虫体はこの宿主の腸管内で，少くとも3日間は生存し，吸溝が発達することを報告した (Furukawa, 1968)．このことは，Plerocercoid から成条虫への発育を誘発する要因として，胆汁以外のものがあることを示すものと考えられるが，この点をさらに追求するために，数種の胆汁酸溶液中で Plerocercoid を短期間培養し，胆汁酸の直接的影響を観察した．

実験に供した胆汁酸は，Sodium deoxycholate, Sodium glycocholate, Sodium cholate および Sodium taurocholate である．各試薬は Earle 液に溶解し，気相を 5% CO_2-95% N_2 として，37°Cで無菌的培養を行なつた．

上記の胆汁酸は，いずれも Plerocercoid の体部を切断あるいは崩壊した．頭部は崩壊されず，しばしば頭部先端が醜出したが，吸溝が発達することなく死滅した．

Plerocercoid の生存率は，胆汁酸の種類と濃度によつて差が認められた．すなわち，Sodium deoxycholate と Sodium glycocholate は，比較的低濃度，短時間で虫体を死滅させたが，他の胆汁酸は大きな効果を示さなかつた．このことから，deoxycholic acid または glycine と結合した胆汁酸をある濃度以上に含む胆汁は，その宿主の腸管内における虫体の発育に，有害な効果をおよぼすことが考えられる．この観点からマンソン裂頭条虫の宿主のなかで，その胆汁成分が知られているものについて比較してみると，終宿主のイヌあるいはネコの胆汁にはこれら有害と思われる胆汁酸の量が少ないことが注目される．一方，第2中間宿主についてみると，その胆汁成分は種によつて大きく異なり，胆汁酸に関して，第2中間宿主に共通する特質を見出すことができなかつた．したがつて，本条虫の宿主特異性発現における宿主側の要因として，胆汁酸は必ずしも決定的役割をおよぼすものではないと考えられる．