

隠岐産 *Trichobilharzia physellae* に関する研究

(4) Cercaria 育成に関する研究

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特 別 掲 載

緒 言

隠岐諸島に棲息するカモ類には、屢々 *Trichobilharzia* 属住血吸虫の寄生を認め、特に *T. physellae* (Talbot, 1936) の寄生の多きことは、既に報告した(第1篇参照)又、隠岐産モノアラガイ *Lymnaea japonica* には、*Trichobilharzia* 属住血吸虫の中間宿主として Ocellata 型 cercaria が繁殖し、しかも、その75%まで *Cercaria physellae* の寄生を認めることは、第2篇に述べた通りである。この種の住血吸虫成虫及び cercaria は、当初小田 (1958) により検討されたが、形態的の同定が保留されていた。

私は、前報三篇の研究により隠岐島産小田記載の住血吸虫 cercaria を *T. physellae* と同定し、且つ、形態的のみならず、成虫育成実験により種の同定を確認したのである。

しかしながら、鳥類住血吸虫の成虫育成実験は、大磯 (1927), Brumpt (1931), Mc Mullen & Beaver (1945) Neuhaus (1951), Macy (1955) により行われているが種属明瞭な miracidium より cercaria 育成実験を試みるものは、Neuhaus (1951), Macy (1955) 等を数えるに過ぎない。

形態的に類似性の多い Ocellata 型住血吸虫 cercaria の種別決定上、私は、先に cercaria の成虫育成法の有力なることを認めたが、こゝに、miracidium の cercaria 育成による種属同定法の意味を検討すべく本実験を企てた。

私は、隠岐産カモより検出した *T. physellae* の卵子の miracidium の育成実験を試み、育成せる cercaria の形態的特徴を調べ、果してこれが種属同定に決定的な意義を有するや否かを検討した。

実験材料及び実験方法

隠岐産カルガモ *Anas poecilorhyncha zonorhyncha*

本研究は文都省科学研究費の一部によつて行われたことを記す。

の腸粘膜より *Trichobilharzia physellae* の成熟卵子を検出、分離し、pH 6.8~7.2, 水温 20~25°C の水を加え観察し游出する miracidium を直ちに中間宿主感染実験に用いた。

実験用中間宿主には、当研究室において累代飼養の無感染モノアラガイを使用した。

殻径 7~10mm の大きさの貝6箇を、5月下旬、室温 25°C, 水温 20~25°C, pH 6.8~7.0 の条件の水中で miracidium と接触せしめ、貝1箇につき、2~4隻ずつ自働的に侵入させた。

感染貝は、20~25°C の恒温水槽に長期飼養し、25日目に3箇の貝より自然に游出する cercaria を認めたので、これを生鮮状態のままに観察する一方、90~100°C に加熱せる 10%ホルマリンで固定した全形標本につき cercaria の各部位を計測した。

実験成績

cercaria の形態

体部、幹尾及び岐尾に区分する。

体部は、長さ 0.296 ± 0.028 mm, 幅 0.049 ± 0.011 mm, 体表は cuticula により覆われている。前部には頭部器官があり、長さ 0.101 ± 0.014 mm, 幅 0.041 ± 0.005 mm である。その前端の体表には、両側に近く、五対の小棘が認められた。腹吸盤は、その径 0.026 ± 0.005 mm 腹面中央に位置し、体部後端までの距離 0.093 ± 0.010 mm, 稍々後方に向け突出している。

毒膚は、腹吸盤の前後に、前二対、後三対と計五対あり、前二対の細胞体内には、不透明顆粒が多数認められた。毒腺導管は、背腹両側に屈曲迂折しつゝ体前端両側の小棘の部に開いている。

眼点は、腹吸盤の前背側に近く存在する。体部両側体表には、八対の感覚突起が認められる。

食道は、頭部器官部の体表腹側中央の口に始まる細い

Table 1. Comparative table of scdistosome cercaria by four Authors

Part Author (year)	Body		Tail stem		Tail furca		Head organ		Distance from ventral sucker end of body.	Diameter of ventral sucker.
	L.	W.	L.	W.	L.	W.	L.	W.		
<i>Cercaria physellae</i> (Talbot, 1939)	0.265	0.060	0.374	0.040	0.196	0.032	0.095	0.038	0.080	0.029
<i>T. physellae-like</i> cercaria (Oda, 1958)	0.268	0.092	0.356	0.049	0.232	0.021	0.091	0.058	0.090	0.030
Cercaria of A. type (Tanaka, 1960)	0.281	0.057	0.347	0.035	0.212	0.024	0.086	0.044	0.096	0.017
Cercaria of aquiered by growth-experiment (Tanaka, 1960)	0.296	0.049	0.306	0.032	0.208	0.020	0.101	0.041	0.093	0.026

L.Length. W.Width.
(in millimeter) Measurement on the specimens fixed with hot 10% formalin solution.

管で、眼点直後中央で二条の短い腸原基に連なる。
排泄系は、終末細胞が頭部器官と腹吸盤との間に三対腹吸盤と体後端までの間に三対、何れも体両側の背側方に対称的に分布する。各終末細胞は、細排泄管により前後二対の排泄管につながり、腹吸盤の両側後方にて一對の排泄主管に合流する。尚、幹尾前端に一對の終末細胞があり、細排泄管は、体側を貫いて後排泄管に連なっている。これ等の終末細胞は、 $[(3+3)+1]2$ の式により示される。

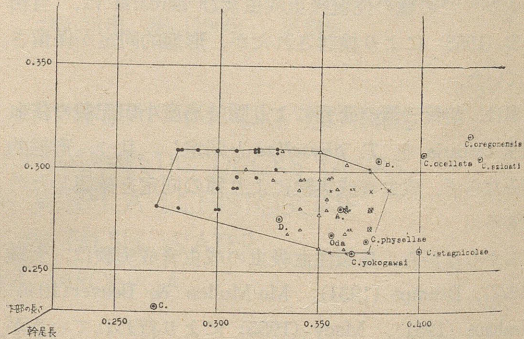
排泄主管の起始部の管壁には、二対の Cilien が認められる。左右の主管は、毒腺細胞の間を迂曲後走して体後端にある一つの排泄腔に入り、更に再び左右二条の管に分れて幹尾に入り、その直後、一条となつて幹尾中心を貫いて幹尾後端に至り、再び二条に分れて、両岐尾に入り、岐尾尖端に開口している。

幹尾は、長さ $0.306 \pm 0.028\text{mm}$ 、幅 $0.041 \pm 0.005\text{mm}$ 岐尾の長さ $0.202 \pm 0.002\text{mm}$ 、幅 0.020 ± 0.002 である。幹尾、岐尾共に表面に多数の小棘が認められ、岐尾には矢状面に一致して、うすい鰭膜が全長に亘つて認められた。

生態：貝より游出せる cercaria は、水面近くを、その幹尾、岐尾を激しく動かして体部方向に前進する。特に光を与えた時、その方向に進む趨光性を有する。又、一部は、水面近く、岐尾を上にして静止するか、試験管内では、管壁又は水面に腹吸盤を接して「く」字型に静止している。

上記、cercaria の形態的特徴の一部として、体部の長さ
と幹尾の長さの函数をグラフに表記するに表IIの如き

Table 2. 実験的育成による *C. physellae* の計測
値関及文献記載 cercaria の比較 (体部の
長さ及幹尾の長さをグラフにする)



単位：mm

実線を以て表現される。

T. physellae の育成 cercaria と、隠岐産 cercaria の比較：*T. physellae* の育成 cercaria と Talbot 記載の *C. physellae*、小田 *physellae* 類似 cercaria 及び私の Cercaria A の各計測値を比較するに Table. 1 のに示す如くその平均値に大差なきことが知れる。従つて計数上、四種の同種性が明示された。

総括及び考按

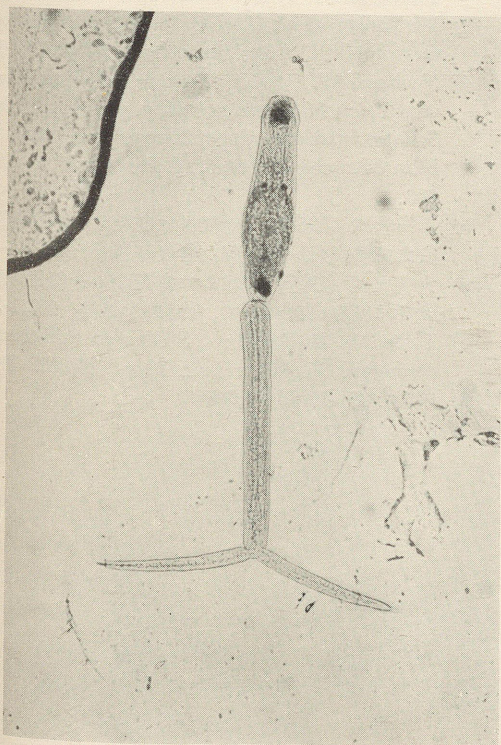
隠岐産カモに寄生する *Trichobilharzia physellae* の miracidium をモノアラガイに感染せしめ飼養した実験に於いて感染後 25 日目に岐尾 cercaria の自然游出を認めた。この cercaria は、隠岐産モノアラガイに自然に寄生する Ocellata 型 cercaria 群中、私の同定した (第2, 3 篇参照) *Cercaria physellae* と一致する この実験成



(1)



(2)



(3)

附図説明

- 写真 1. *T. physellae* の卵子 (40×10)
 2. 水中に遊出せる miracidium (40×10)
 3. 実験的に育成せる cercaria ホルマリン固定 (10×40)

績は、当然の結果と言える。

然し乍ら、この cercaria の各部位を $90^{\circ}\sim 100^{\circ}\text{C}$ 、加熱 10%ホルマリン固定後計測せる数値は、Talbot 所報の数値に比して著しく広範なるは、注目すべき所見である。*C. physellae* の発見者 Talbot は、Ocellata 型 cercaria の分類は、形態的には困難で計測値の差によつてのみ可能であると述べたが、私が *T. physellae* の miracidium より育成した cercaria は、何れも同一貝に發育した cercaria でありながら、計測値の範囲が著しく大きく、文献上、既知の Ocellata 型の諸 cercaria の内の一定種は、私の育成 cercaria の計測値のグラフに於ける実線内に入る。即ち、*C. physellae* 小田(1958)、*physellae* 類似 Cercaria、私の Cercaria A、Cercaria D がこの範囲内に見られ、Cercaria B、Cercaria C、Talbot (1936) 記載の *C. elvae* Miller, 1931、*C. oregonensis* McFarlane & Macy, 1949、*C. szidati* Neuhaus 1951、*C. yokogawai* Oiso, 1927 及び *C. stagnicola* Talbot, 1936 は実線外に出ている。この事實は、Talbot の計測値の差による類別法による Ocellata 型 cercaria の分類は、正確を期し難いことを物語っている。Ocellata 型 cercaria の形態及び計測による分類は、こゝに根本的に再検討すべき事實に直面した。従つて、Ocellata 型 cercaria に於いては、特に生活史追求実験による生物学的同定の重要性を私は指摘したい。即ち、cercaria 形態学の現況に於いて cercaria の種別決定は、Ocellata 型 cercaria 群では必ず cercaria より成虫、miracidium より cercaria への發育史的關聯性を明らかにし、成虫の種別に従つて同定すべきである。

結 論

- 1) 隠岐産 *T. physellae* の卵子より得たる miracidium をもつて、実験的にモノアラガイ *Lymnaea japonica* を中間宿主とする cercaria を育成した。
- 2) 本実験による *T. physellae* の miracidium より

育成された cercaria は、隠岐産モノアラガイに寄生する Cercaria A と同一種であることが確認された。

3) 育成 cercaria の形態の数値は、Talbot の記載の *Cercaria physellae* の形態の数値より変動幅が極めて大である。

従つて、Ocellata 型 cercaria の種属同定には、形態的特徴のみならず、發育史追求による成虫との關連性を決定することが重要である。

稿を終えるに當り、終始御懇切なる御指導と御校閲を賜つた恩師田部浩教授に深謝す。

尙、本研究は、第28回日本寄生虫学会に於いて、要旨を報告した。

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STUDIES ON TRICHOILHARZIA PHYSELLAE IN OKI ISLANDS

4. EXPERIMENTAL INFECTION OF THE SNAILS,
LYMNAEA JAPONICA WITH THE MIRACIDIA
OF TRICHOILHARZIA PHYSELLAE

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Despite the large amount of experiments on the development of avian schistosome cercariae to the adult in host animals, a few data on the experimental infection of the intermediate host with clearly identified miracidia was available and its successful development to cercariae in the host is poorly understood. This study, the fourth in a series, was made to investigate the miracidia collected from the eggs of *T. physellae*. The results obtained were summarized as follows:

1) The cercariae obtained from experimentally infected *Lymnaea japonica* with miracidia hatched from eggs of *T. physellae* was identified with those from naturally infected *L. japonica* in Oki Islands.

2) Values obtained by measuring each part of cercarial body was more variable than those of *Cercaria physellae* reported by Talbot (1936). It is, therefore, very important to obtain the adult specimens by means of experimental infection with unknown cercariae when identifying

COMPARATIVE STUDIES ON THE ANTHELMINTIC EFFECT OF BEPHENIUM HYDROXYNAPHTHOATE, TETRACHLORETHYLENE, 1-BROMO- β -NAPHTHOL AND 4-IODOTHYMOL AGAINST THE HUMAN HOOKWORM

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Tetrachlorethylene, since its discovery by Hall and Shillinger in 1925, has been used generally in the treatment of hookworm infections. Usually large doses of saline purge have been taken with the anthelmintic because of fear for its toxicity. However, Carr *et al.* (1954) have indicated that tetrachlorethylene was more effective in removing worms and provoked less complaint in the patients when it was not followed by a saline purge. Afterwards Yoshida *et al.* (1952, 1956), Komiya *et al.* (1956), Pinto *et al.* (1956) and Yanagisawa (1957) re-examined this method of the sole application of the drug without using a purge and certified the validity of the claim. On the other hand, Miura *et al.* (1952, 1953) have synthesized 4-bromo- α -naphthol and 1-bromo- β -naphthol and reported on their anthelmintic effects against nematodes in the toad. Iwata (1953) later showed that both of 4-bromo- α -naphthol and 1-bromo- β -naphthol had high activity against human hookworms while possessing low toxicity on the human body. Currently, tetrachlorethylene and 1-bromo- β -naphthol have been used commonly in the treatment of hookworm infections in Japan.

Most recently, the authors were given a new type of anthelmintic, bephenium hydroxynaphthoate ("Alcopar"), by Dr. Goodwin of the Wellcome Laboratories of Tropical Medicine, London. Goodwin *et al.* (1958) in Ceylon and Young *et al.* (1958) in America have carried out clinical trials with bephenium salts against

Necator americanus, and Copp *et al.* (1958), Burrows (1958) and Kobayashi *et al.* (1959) have reported on the anthelmintic effect of bephenium salts against *Ancylostoma caninum*, while Rogers (1958) has studied on the excretion of bephenium salts into urine of human beings.

The present paper describes the results of clinical trials with bephenium hydroxynaphthoate compared with tetrachlorethylene, 1-bromo- β -naphthol and 4-iodothymol against hookworms in a Japanese farm village.

Materials and Methods

1. The location of the clinical trials.

A village of Kumihama-cho of Kyoto Prefecture was selected for the present trials. Most of the inhabitants of this village were engaged in agriculture, especially in rice-crop and fruit-culture. In August, 1959, patients, whose stools were positive for hookworm ova on direct smear method seeking up to three specimens per each, were selected and treated under field conditions. Those patients were divided into ten groups (A-J group) at random to be subjected to several ways of treatment.

2. Anthelmintics and dosages.

A-group: Bephenium hydroxynaphthoate ("Alcopar"; a preparation of the Burroughs Wellcome & Co.), 4.0g in terms of base (8.0g as "Alcopar" preparation) for adults, with proportionately smaller doses for children, followed by a saline purge (25g of magnesium sulfate

with about 500cc of water).

B-group: Bephenium hydroxynaphthoate, 4.0g without any purge before or after treatment.

C-group: Bephenium hydroxynaphthoate, 2.5g in terms of base (5.0g as "Alcopar" preparation) for adults with a saline purge.

D-group: Bephenium hydroxynaphthoate, 2.5g without any purge.

E-group: Tetrachlorethylene ("Tetren"; a preparation of the Kuroda Pharmaceutical Co., Ltd.), 4.5g for adults with a saline purge.

F-group: Tetrachlorethylene, 4.5g without any purge.

G-group: 1-Bromo- β -naphthol ("Wormin"; a preparation of the Toyamakagaku & Co., Ltd.), 8.0g for adults (12.0g as "Wormin" preparation) with a saline purge.

H-group: 1-Bromo- β -naphthol, 8.0g without any purge.

I-group: 4-Iodothymol ("Thymolan"; a preparation of the Chugai Pharmaceutical Co., Ltd.), 6.0g for adults (12.0g as "Thymolan" preparation) with a saline purge.

J-group: 4-Iodothymol, 6.0g without any purge.

These anthelmintics were given in the morning to the patients having fasted since the previous evening, and no food was allowed until two or three hours after the dose. These anthelmintics were administered in two doses of an equal amount with an interval of about 30 minutes in order to minimize the irritation of the stomach.

3. Judgment of anthelmintic effect of these medicines.

The anthelmintic effect of each medicine was assessed by the following methods.

(a) When the patients were treated with these four anthelmintics followed by the saline purge, all bowel movements within 24 hours after the drug dosage were collected and washed to obtain all recoverable worms. The recovered worms were counted and the species identified.

(b) On the 14th day after the treatment, a detailed feces examination was carried out by such methods as direct smear, floatation by

saturated NaCl and egg culture. And the rate of stools negative for hookworm ova was calculated (herein designated as cleared-rate). In Japan, such methods as mentioned above are usually employed to determine the effects of anthelmintics.

In this point, we must employ an exact method for feces examination after the treatment to prevent errors in evaluation of its effectiveness. In order to make clear the effect of a new anthelmintic, it is important to compare it with other anthelmintics which had been examined sufficiently in the past.

Results

1. Incidence of hookworm infestation and species of hookworms on the experimental field.

The feces examination was carried out on 859 inhabitants of the experimental field mentioned above in August, 1959. Intestinal parasitic helminths revealed and their incidences are shown in Table 1. By many recent researches the rate of hookworm infestation in Japanese farm villages shows an average of 20-30%.

Table 1. Incidence of intestinal parasitic helminths in the experimental field (Kumihama-cho, Kyoto Prefecture)

No. examined	Hookworm		Roundworm		Whipworm	
	No.	%	No.	%	No.	%
859	283	32.9	258	30.0	257	29.9

We have identified the species of hookworm, *Ancylostoma duodenale* (*A. d.*) and *Necator americanus* (*N. a.*) which were isolated from stools of the patients after the treatment. As is shown in Table 2, it was revealed that there were *A. d.* simple infection in 22 cases (32.8%), mixed infection of *A. d.* and *N. a.* in 18 cases (26.9%) and *N. a.* simple infection in 27 cases (41.3%). On the other hand, the number of isolated worms were 278 (20.7%) *A. d.* and 1062 (78.3%) *N. a.*. In Japan, both species of the hookworm, *A. d.* and *N. a.*, are distributed widely. It was considered that *N. a.* was distributed more predominantly than *A. d.* in the

present experimental field.

2. The effect of these anthelmintics in the treatment of hookworm infection.

The rates of expulsion of hookworms within 24 hours after the treatment by these anthelmintics with the saline purge, are shown in Table 3. The rates of stools negative for hookworm ova (cleared-rate) by the subsequent stool examination on the 14th day after the treatment are shown in Table 4.

from the stools was not carried out in cases not given a purge. Hookworm ova were negative in 11 out of 22 cases treated (50.0%). We then tested the significance of the difference of cleared-rates between cases with and without the purge by the χ^2 -test (level of significance: 5%). No significant difference of cleared-rates between A and B-groups was seen ($\chi^2=0.02$).

C-group: Bephenium hydroxynaphthoate 2.5g with a purge: In 10 out of 19 cases treated (52.5%), hookworms were recovered, and in 7

Table 2. Proportion of the infestation of *Ancylostoma duodenale* and *Necator americanus* in the experimental field

Cases		Total number of expelled worms	Average number per case	Min.-Max. number per case
A. d.* (Simple infection)	22 (32.8%)	A. d. 278 (20.7%) N. a. 1062 (79.3%)	A. d. 6.9 N. a. 23.6	A. d. 1-66 N. a. 1-160
A. d.+N. a.** (Mixed infection)	18 (26.9%)			
N. a. (Simple infection)	27 (40.3%)			

* *Ancylostoma duodenale*
** *Necator americanus*

Table 3. Results of worms expelled with these anthelmintics

Anthelmintics (Dosage)	No. of cases treated	No. of cases with worms expelled	<i>Ancylostoma duodenale</i>			<i>Necator americanus</i>		
			No. of cases	No. of worms		No. of cases	No. of worms	
				Min.-Max.	Average		Min.-Max.	Average
Bephenium hydroxynaphthoate (2.5 g)*	19	10 (52.5%)	5	2-10	4.6	6	1-37	9.3
Bephenium hydroxynaphthoate (4.0 g)*	17	12 (70.3%)	8	1-3	1.7	8	1-140	28.7
Tetrachlorethylene (4.5 g)	21	15 (71.4%)	10	1-66	11.3	9	2-160	43.7
1-Bromo- β -naphthol (8.0 g)	19	16 (84.2%)	8	1-36	13.2	13	1-77	23.3
4-Iodothymol (6.0 g)	22	14 (63.7%)	9	1-5	2.3	9	1-52	8.7

* Dose in terms of base.

A-group: Bephenium hydroxynaphthoate 4.0g with the purge: Hookworms were recovered from 12 out of 17 patients (70.3%) who were treated with this drug, and the detailed data concerning the worm species are shown in Table 3. Hookworm ova were negative in the stools of 9 out of 17 patients treated (52.9%).

B-group: Bephenium hydroxynaphthoate 4.0g without the purge: Collecting of hookworms

out of 19 cases treated (36.8%) hookworm ova were negative.

D-group: Bephenium hydroxynaphthoate 2.5g without the purge: In 11 out of 22 cases treated (50.0%) hookworm ova were negative in the stools. By the χ^2 -test, a significant difference of cleared-rates between the C and D-groups was not seen ($\chi^2=1.35$).

E-group: Tetrachlorethylene 4.5g with the

Table 4. Comparison of the rates of stools negative for hookworm ova (cleared-rate) after treatment with these anthelmintics

Group	Anthelmintics and Dose	No. of cases treated	No. of cases egg negative	Cleared-rate
A	Bephenium hydroxynaphthoate 4.0 g* with purge	17	9	52.9
B	Bephenium hydroxynaphthoate 4.0 g* alone	22	11	50.0
C	Bephenium hydroxynaphthoate 2.5 g* with purge	19	7	36.8
D	Bephenium hydroxynaphthoate 2.5 g* alone	22	11	50.0
E	Tetrachlorethylene 4.5 g with purge	20	6	30.0
F	Tetrachlorethylene 4.5 g alone	17	8	47.1
G	1-Bromo- β -naphthol 8.0 g with purge	16	10	62.5
H	1-Bromo- β -naphthol 8.0 g alone	60	46	76.6
I	4-Iodothymol 6.0 g with purge	22	4	18.2
J	4-Iodothymol 6.0 g alone	21	5	23.8

* Dose in terms of base

purge: In 15 out of 21 cases treated (71.4%), hookworms were recovered and in 6 out of 20 cases treated (30.0%), stools examined for hookworm ova were negative.

F-group: Tetrachlorethylene 4.5 g without the purge: In 8 out of 17 cases treated (47.1%), hookworm ova were negative. The χ^2 -test showed that a significant difference of cleared-rates between the E and F-groups was not seen ($\chi^2=1.98$).

G-group: 1-Bromo- β -naphthol 8.0 g with the purge: In 16 out of 19 cases treated (84.2%), hookworms were recovered, and in 10 out of 16 cases treated (62.5%), stools examined for hookworm ova were negative.

H-group: 1-Bromo- β -naphthol 8.0 g without the purge: In 46 out of 60 cases treated (76.6%), stools for hookworm ova were negative. By the χ^2 -test, a significant difference of cleared-rates between the G and H-groups was not seen ($\chi^2=2.19$).

I-group: 4-Iodothymol 6.0 g with the purge: In 14 out of 22 cases treated (63.7%), hookworms were recovered from the stools and 4 out of 22 cases treated (18.2%) showed stools in which hookworm ova were negative.

J-group: 4-Iodothymol 6.0 g without the purge: In 5 out of 21 cases treated (23.8%), stools for hookworm ova were negative. We

could not find any significant difference of cleared-rates between the I and J-groups by the χ^2 -test ($\chi^2=0.69$).

According to the results mentioned above, no significant differences of cleared-rates between the purge and no purge groups could be found by the χ^2 -test with any of the anthelmintics.

Next, the cleared-rates of the purge and no purge groups were summed up for each anthelmintic, and it was calculated whether any significant difference existed between bephenium hydroxynaphthoate and the others. The results obtained were as follows; No significant difference of cleared-rates between bephenium hydroxynaphthoate and tetrachlorethylene was seen ($\chi^2=0.61$). While bephenium hydroxynaphthoate was proved to be less efficient than 1-bromo- β -naphthol, it was more efficient than 4-iodothymol, both to a significant degree ($\chi^2=12.28$ and $\chi^2=7.28$, respectively).

3. The difference of resistance between *Ancylostoma duodenale* and *Necator americanus* against these four anthelmintics.

Hitherto, some authors (Sopar, 1926; Yoshida, 1956 etc.) have pointed out that tetrachlorethylene was more effective on *N. a.* than on *A. d.* Goodwin *et al.* (1958) reported on the effect of bephenium salts against *N. a.* in Ceylon. In

the present study, we considered the problem of whether there was any difference of resistance between *A. d.* and *N. a.* against these four anthelmintics. As is shown in Table 5, *A. d.* seems to be less resistance than *N. a.* against bephenium hydroxynaphthoate. On the contrary, *N. a.* seems to be less resistant than *A. d.* against tetrachlorethylene and 4-iodothymol. Both species showed an almost equal resistance against 1-bromo- β -naphthol.

4. Effect of bephenium hydroxynaphthoate in the treatment of roundworm infections.

Tetrachlorethylene has some effect upon the young roundworm, but it is nearly ineffective to the adult roundworm. Table 6 shows the effect against the intercurrent *Ascaris* infections in the patients treated for hookworms.

The cleared-rate which was calculated by the presence or absence of eggs at follow-up feces

examination, was 50.0% and 57.8% with the doses of 2.5 g and 4.0 g of this drug, respectively. This drug has good efficacy against roundworms as Goodwin *et al.* (1958) has already pointed out.

5. Side-effects of these anthelmintics.

We questioned the patients who received the present treatment about side-effects and, if any, their degrees. Fig. 1 shows the kinds and rates of side-effects. Generally speaking, no remarkable difference between the purge and no purge groups was found. With tetrachlorethylene, however, in the no purge group, the degree of each side-effect may be lower than in the purge group. In the case of bephenium hydroxynaphthoate, it seems that the degree of each side-effect did not increase when the dose was increased from 2.5 g to 4.0 g. Bephenium hydroxynaphthoate showed side-effects in a con-

Table 5. Difference of resistance between two species of hookworm against these anthelmintics

Anthelmintic	Species of hookworm	No. of cases treated	No. of cases egg negative	Cleared-rate
Bephenium hydroxynaphthoate	<i>A. d.</i> *	12	7	58.2
	<i>N. a.</i> **	19	7	36.8
Tetrachlorethylene	<i>A. d.</i>	12	4	33.3
	<i>N. a.</i>	11	6	54.5
1-Bromo- β -naphthol	<i>A. d.</i>	7	6	85.8
	<i>N. a.</i>	8	7	87.5
4-Iodothymol	<i>A. d.</i>	12	2	16.7
	<i>N. a.</i>	8	4	50.0

* *Ancylostoma duodenale*

** *Necator americanus*

Table 6. Effect of Bephenium hydroxynaphthoate against roundworms

Doseage	No. of cases treated	No. of cases egg negative	Cleared-rate
4.0 g with purge*	13	7	53.8
4.0 g without purge*	6	4	66.9
2.5 g with purge*	7	3	42.9
2.5 g without purge*	5	3	60.0
Total	31	17	54.8

* Dose in terms of base.

siderable percentage, but the symptoms were not severe compared with those caused by the other drugs, and disappeared within 1-2 hours. Tetrachlorethylene characteristically produced nervous symptoms such as dizziness, drunkenness and headache, but these symptoms were not so severe, especially when giving the drug without a purge as shown in Fig. 1. In the 1-bromo- β -naphthol cases, the kinds and degrees of side-effects were similar to those of bephenium hydroxynaphthoate. The toxicity of 1-bromo- β -naphthol is considered to be very low by our repeated experiences. Side-effects of 4-iodothymol was not so heavy in general, but this drug produced a marked laxative effect in most of the patients treated even when followed by no purge.

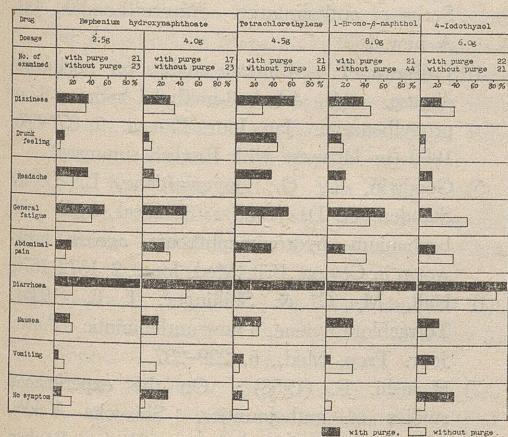


Fig. 1. The kinds and degrees of side-effects of these anthelmintics

Discussion

Tetrachlorethylene has been used for many years in the treatment of hookworm infections and its anthelmintic efficacy has been sufficiently studied by many authors. As Carr *et al.* (1954) pointed out, we have also studied tetrachlorethylene for several years and have concluded that no purge is necessary with this drug (Yoshida *et al.*, 1955, 1956). In the present paper, similar results were obtained with tetrachlorethylene. According to the results of Goodwin *et al.* (1958) on bephenium hydroxynaphthoate, the mean reduction in the hook-

worm egg count with a single dose of this drug were 28% with a dose of 0.5-1.5g, 78% with 2g, and 64% with 2.5-3.0g. No increase in efficacy was seen between 2g and 2.5-3.0g in dose. Furthermore Goodwin *et al.* showed that the effect of three doses of this drug given on the same day, each containing 2g, was similar to that produced by a single dose of 2-3g. Our results also showed a similar tendency as follows: the cleared-rate of 2.5g of bephenium hydroxynaphthoate was 36.8-50.0% and that of 4.0g was 50.0-52.9%, and no significant difference of cleared-rates was found by the χ^2 -test ($\chi^2=0.78$). Later Goodwin *et al.* showed that the anthelmintic effect of bephenium hydroxynaphthoate did not increase by using a purge before or after the dose in the treatment of 132 hookworm infections in the field. We have also shown the ineffectiveness of the purge, not only in bephenium, but in the other three drugs. As we have shown in Table 4, a single dose of 2.5-4.0g of bephenium hydroxynaphthoate was as effective as 4.5g of tetrachlorethylene.

In judgment of the effect of treatment, we did not employ the Stoll's egg count method, but resorted to the cleared-rate method by the detailed feces examination on the 14th day after the treatment. The cleared-rates calculated from Table 2 and Table 3 of Goodwin's paper are 37.0% in three doses each of 2g on one day and 26.3% in dose of 2g daily for 4-7 days. Most recently, Morishita *et al.* (1960) indicated that bephenium hydroxynaphthoate showed a high cleared-rate incidence (nearly 100%) in the treatment of 30 cases of hookworm infection in Japan. Such a difference of cleared-rates among Goodwin *et al.*, Morishita *et al.* and us may be due to the difference of the species of worm and the intensity of hookworm infestation in their experimental fields.

Since the discovery of 1-bromo- β -naphthol by Miura (1952), many authors have reported on the high efficacy of this drug against hookworms (Iwata, 1953; Matsuzaki, 1957; Mai K'ai, 1959; Wang, 1959 etc.). Our results showed that 1-bromo- β -naphthol was the most effective among them. Hayashi *et al.* (1958, 1959) have indicated that 4-iodothymol has an anti-hookworm

effect. Subsequently, Fujisawa (1958), Sasa *et al.* (1958) and Komiya *et al.* (1959) have reported on the anthelmintic efficacy and side-effects of 4-iodothymol. The cleared-rate of this drug against hookworms as reported by these authors ranges from 30 to 50%. By our results obtained, the cleared-rate (21.0% on the average) was the lowest among the anthelmintics tested.

Bephenium hydroxynaphthoate was effective against roundworms. The cleared-rate was 50.0–57.8% with a single doses of 2.5–4.0 g. This rate was very similar to that of Goodwin. For the mass treatment, it would be valuable practically that this drug has anthelmintic efficacy not only on hookworms but upon roundworms.

Summary

Bephenium hydroxynaphthoate which was recommended as a new anthelmintic by Goodwin *et al.* (1958) was compared with tetrachlorethylene, 1-bromo- β -naphthol and 4-iodothymol in the treatment of hookworm infections in Kumihama-cho, Kyoto Prefecture, a farm village of middle Japan. The hookworm incidence in the experimental field was 32.9%. In calculating the number of expelled hookworms. *Ancylostoma duodenale* and *Necator americanus* were 278 (20.7%) and 1,062 (79.3%), respectively.

The anthelmintic effect against hookworms with a single dose of 2.5 or 4.0 g of bephenium hydroxynaphthoate was approximately equal to that of a single dose of 4.5 g of tetrachlorethylene. Bephenium hydroxynaphthoate was inferior to 1-bromo- β -naphthol and superior to 4-iodothymol in the anthelmintic effect. The side-effects of bephenium hydroxynaphthoate were seen in a considerable percentage, but not as toxic and not in so many cases as compared with the other three anthelmintics. It is unnecessary to take any purge with these anthelmintics from the view-point of their efficacy and side-effects. It was reconfirmed that bephenium hydroxynaphthoate has a marked effect against roundworms.

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