

# CHEMOTHERAPY OF PARAGONIMIASIS WITH BITHIONOL

## 1. EXPERIMENTAL CHEMOTHERAPY ON THE ANIMALS INFECTED WITH *PARAGONIMUS WESTERMANI* OR *P. OHIRAI*.

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### Introduction

Emetine hydrochloride was used for the first place for chemotherapy of human paragonimiasis by Ikeda (1915). Yokogawa *et al.* (1934-1941) found that Emetine hydrochloride combined with Prontosil was much more effective against human paragonimiasis than the use of Emetine hydrochloride alone.

This combined method is now widely used for chemotherapy of paragonimiasis in Japan. Recently Chung *et al.* (1954) reported that Chloroquine (Resochin) was quite effective for this disease. Kitamoto *et al.* (1957) and several other investigators in Japan tried this drug for paragonimiasis and concluded that Chloroquine was not so effective as Emetine hydrochloride with sulfonamids during such short period as two or three weeks of treatment and it produced some side-effects.

Since the screening method of the effective drugs for paragonimiasis by means of the maintaining technique of excysted metacercariae of *Paragonimus westermani in vitro* has been established by M. Yokogawa *et al.* (1955-1956), several drugs which showed strong killing effects on the larvae *in vitro* were found in our laboratory. The direct effects of several drugs against the larvae *in vitro* were as shown in Table 1.

Bithionol seemed to be the most effective drug for paragonimiasis among those drugs which

were chosen by the screening method.

The present study was conducted to use Bithionol for the chemotherapy of the experimentally infected animals with *Paragonimus westermani* or *P. ohirai*.

Dogs or cats infected with *P. westermani* are usually used as the experimental animals for the chemotherapy of paragonimiasis. However, it is quite difficult to use a large number of these animals at the same time. The authors here attempted to apply many rats infected with *P. ohirai* in place of the infected dogs or cats with *P. westermani*.

It was found that Bithionol could be successfully used for the chemotherapy of paragonimiasis of the animals experimentally infected with *P. ohirai* as well as *P. westermani*.

Table 1. Comparison of killing effects of drugs against excysted metacercariae of *Paragonimus westermani in vitro*

Drugs	LD <sub>50</sub> (Dilution)	
	24 hours	48 hours
Stibnal	11,500	17,000
Emetine	230,000	250,000
Atabrine	323,000	420,000
Chloroquine (Resochin)	389,000	470,000
Bithionol	1,390,000	

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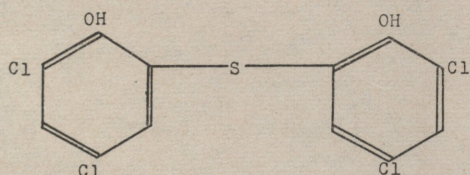
The authors (1960) also used this drug clinically on 13 cases of human paragonimiasis and obtained the excellent results.

The present paper dealt with only the results of the animal experiment.

### Materials and Methods

#### Drug used.

The drug used for this trial, Bithionol is a compound with such a structure as shown in Fig. 1; it is a tasteless, odorless and white crystalline powder and its MP is 128°C; SG is 1.73 (25°C): it is poorly soluble in water, i. e., 0.0004% at 25°C, but soluble in carboxymethylcellulose (CMC), an organic solvent at 72% (25°C). Since this drug has a sterilizing effect on the skin, it has been used under the commercial name, Actamer (Monsanto Co.), in the United States as an ingredient of soap or cosmetics (Shumard *et al.*, 1953). In this country this agent was found, for the first time, to have an excellent anti-helminthic effect on the chicken tape worm, *Raillietina kashiwarensis* (Sawada, 1957) and the liver fluke of cattles, *Fasciola hepatica*, (Ueno *et al.*, 1959) and this drug is now put out by the Tanabe Seiyaku Co., Ltd. as Bitin, an anti-helminthic drug for animal use. However, it has never been used for the lung fluke, *Paragonimus* sp. In this experiment the plate-like tablets or powder of Bitin were administered per os to the experimental animals.



2,2'-thiobis (4,6-dichlorophenol)

Fig. 1. The structural formula for Bithionol (Actamer, Bitin)

#### Experimental animals.

Four dogs of 8-10 kg of body weight were infected with 20-123 metacercariae of *P. wester-*

*mani*. Bitin was administrated during the various periods from 79 days to 318 days after infection. Four dogs were all sacrificed after the treatments and examined pathologically and parasitologically.

Adult rats of about 150 gr of body weight were infected with 6-10 metacercariae of *P. ohirai*. Since some of the adult worms of *P. ohirai* in the worm-cysts of the lungs of the rats begin to die naturally within 2-3 months after infection in general, the same number of the infected rats without treatment as that of the treated animals were also sacrificed at the same time as the control.

#### Toxicity-test of Bitin on animals.

Bitin has been applied in a single dose for the animals in Japan as shown in Table 2. However,

Table 2. Anthelmintic dose of Bitin on animals

Animals	Doses	Authors
hens	100 mg/kg.	(Sawada, 1958)
"	100-200 mg/kg.	(Kondo, 1958)
sheep	50-300 mg/kg.	(Ueno, 1959)
cattle	30-100 mg/kg.	(Ueno, 1959)
horse	5- 50 mg/kg.	(Sasa, 1959)
"	15 mg/kg. X 5	(Sasa, 1959)
goat	100 mg/kg.	(Matsuzaki, 1959)
puppy	1- 3 gr/kg.	(Tanabe, Co., 1959)

#### Appendix:

Median Lethal Doses (LD<sub>50</sub>) of Bitin on the animals. (Tanabe Institute)

rat : 5.77 gr/kg.  
 mouse : 1.428 gr/kg.  
 rabbit : 2.1-4.7 gr/kg.

it has been known that the chemotherapy for paragonimiasis with Emetine hydrochloride, chloroquine or others is needed to continue at least for two or three weeks. Bitin was not seemed to be an exception. Preliminary toxicity-test of Bitin on mice and rats were carried out as follows.

70 mg/kg - 300 mg/kg of Bitin were given orally to mice and rats daily, every other day or every two days respectively for 5-30 times, and toxicity was examined histopathologically on

liver, kidney, spleen, ovary, testis, brain, lung, and intestine of these animals.

The results obtained in mice and rats are as shown in Table 3 and 4.

Any remarkable changes were not proved in mice and rats which were given daily 70 mg/kg of Bitin for 5-8 times as well as the control.

As the pathological changes ascites and moderate degenerations of liver, hyperplasia of the follicles in spleen and catarrhal phenomena in intestines were found in mice which were given daily 150 mg/kg of Bitin for 5 times. These pathological changes were not so remarkable even in the rats given 300 mg/kg of Bitin daily for 30 times. Any pathological changes were not found in lungs, brain and reproductive organs in all cases.

Any remarkable reduction of body weight was not noticed.

From the above-mentioned results, the toxicity

of Bitin was not so serious even in the continuous use. The conclusion was reached that it will be safe to give 70 mg/kg - 100 mg/kg of Bitin every other day.

### Evaluation of the Efficacy of Bitin

The method of evaluation of the efficacy of the drug for paragonimiasis has not been established. In the present study the special attentions for the criterion of the effect of the drug were paid on the next three matters as described under.

1. Variation of the number of eggs in feces (E. P. D.) during the course of treatment.

E. P. D. of the experimental dogs and rats were examined by Stoll's eggs-counting technique (modified method, 1926) and A. M. S. III centrifugation method.

Trble 3. Toxicity-test of Bitin given orally on animals

Mice :

Sex of animals	Method of administration	Dose	Body weight (grams)		Pathological changes						
			Before admin.	After admin.	liver	kidney	intestine	spleen	lung	brain	ovary or testis
♂ ♀ ♀	daily	70 mg/kg	28	27	+	-	-	-	-	-	-
		" X 5	23	23	-	+	+	+	-	-	-
		"	26	24	++	+	-	+	-	-	-
♂ ♀ ♀	every other day	"	27	27	-	-	+	-	-	-	-
		" X 8	24	23	+	+	-	+	-	-	-
		"	28	29	-	+	+	+	-	-	-
♂ ♀ ♀	every two days	"	20	22	+	-	-	-	-	-	-
		" X 5	21	20	+	-	+	-	-	-	-
		" X 8	30	31	-	-	-	+	-	-	-
♂ ♀ ♀	daily	150 mg/kg	26	26	-	+	+	+	-	-	-
		" X 5	28	27	++*	+	++	++	+	+	-
		"	27	25	++	+	++	++	++**	+	-
♂ ♀ ♀	every other day	"	23	21	+	+	+	-	+	+	-
		"	24	24	+	-	+	+	+	+	-
		"	23	23	-	+	+	++	-	-	-
♂ ♀ ♀	every two days	"	26	27	+	+	-	+	-	-	-
		"	27	27	+	+	-	+	-	-	-
		"	25	28	-	-	+	-	-	-	-
♂ ♀ ♀	control		26	27	+	-	-	+	-	-	-
			25	23	-	-	+	-	-	-	-
			19	19	-	+	-	+	-	-	-

+ : slight changes  
\* : ascites

++ : moderate changes  
\*\* : congestion

Table 4. Toxicity-test of Bitin given orally on animals

Rats :		Dose	Body weight (grams)		Pathological changes							Appendix		
Sex of animals	Method of administration		Before admin.	After admin.	liver	kidney	intestine	spleen	lung	brain	ovary or testis			
♂	daily	300 mg/kg	X 18	127	125	++	+	++	+	-	-	-	diarrhea.	
♂			"	X 20	115	103	+	+	-	++	+	-		-
♀			"	X 21	120	110	++	+	++	+	-	-		-
♀			"	X 30	128	132	+	+	+	+	-	-		-
♀			"	"	120	118	+	++	+	+	-	-		
♂	daily	200 mg/kg	X 22	115	92	+	++	++	+	-	-	-	diarrhea.	
♂			"	X 30	124	129	±	+	+	+	-	-		-
♂			"	"	129	107	+	+	-	++	-	-		-
♂			"	"	112	118	+	-	-	+	-	-		-
♀			"	"	120	131	+	+	-	++	-	-		-
♀			"	"	118	121	+	-	-	-	-	-		-
♀			"	"	153	160	++	+	+	-	-	-		
♂	every other day	200 mg/kg	X 30	104	114	+	+	-	+	-	-	-	diarrhea.	
♂			"	"	115	126	+	+	-	-	-	-		
♀			"	"	120	90	++	+	++	+	-	-		-
♀			"	"	100	118	+	+	-	+	-	-		-
♀			"	"	106	122	-	+	-	+	-	-		
♂	daily	150 mg/kg	X 5	117	117	+	+	++	+	-	-	-	diarrhea.	
♀			"	"	139	137	++	+	+	+	-	-		-
♀			"	"	114	112	+	+	+	++	-	-		-
♂	every other day		X 5	119	124	+	+	+	+	+	-	-		
♂			"	"	122	126	-	-	-	-	-	-		-
♀			"	"	123	124	+	+	-	-	-	-		
♂	every two days		X 5	117	116	+	+	-	-	-	-	-		
♀			"	"	139	139	-	+	-	-	-	-		-
♂	daily		X 8	141	138	+	+	+	++	+	-	-		
♀			"	"	118	119	+	+	-	+	-	-		-
♀			"	"	135	135	++	+	-	+	-	-		-
♂	daily	70 mg/kg	X 5	118	120	+	-	-	-	-	-	-		
♂			"	"	123	123	-	+	+	+	-	-		-
♀			"	"	105	108	-	-	-	-	-	-		-
♂	every other day		X 5	124	125	+	+	-	-	-	-	-		
♀			"	"	100	99	-	+	-	+	-	-		-
♀			"	"	156	159	-	-	-	-	-	-		
♂	every two days		X 5	108	107	+	-	-	-	-	-	-		
♀			"	"	130	134	-	+	-	-	-	-		-
♂	control			106	112	+	+	-	+	-	-	-		
♀					124	127	-	-	-	+	-	-		-

+ : slight changes

++ : moderate changes

E. P. D. was examined daily or every other day before, during and after treatment.

## 2. Pathological and parasitological findings at autopsy.

Number and locations of the worm-cysts and worms in the lungs were examined by autopsy. The worm-cysts were examined histopathologically. All the worms removed from the worm-

cysts were maintained in Tyrode's solution at 37°C for 30-60 minutes to examine their vitalities. The worms were then fixed with 10% formalin solution and stained by haematoxylin. The presence of the eggs in uterus or other degenerated changes of the reproductive organs of the worms were observed.

## 3. The survival rate of the worms.

The rates of the number of survived worms recovered in the treated animals at autopsy to the number of metacercariae given were also compared with that of control animals.

The infection rates (detection rate) of the worms to the number metacercariae given to animals are shown in Table 5.

### Results

#### A. Effect of Bitin on the infected dogs with *P. westermani*.

Dog No. I:

The dog No. I infected with 20 metacercariae of *P. westermani* was given 100 mg/kg of Bitin every other day for 5 times beginning 78 days after infection. E. P. D. showing 50,600 before treatment decreased quickly to 30,000 after the 3rd administration, to 3,030 after the 4th administration and to 0 after the 5th administration of Bitin as shown in Figure 2. No eggs were found in feces during the period of 10 days after the last administration of Bitin, but E. P. D. increased again gradually up to 52,140 on the 22nd day after the last administration. The second

Table 5. Infection rate of *Paragonimus westermani* in animals (Yokogawa *et al.*).

Animals used	No. of metacercariae given	No. of animals used	Days from infection to autopsy	No. animals infected	Total no. worms detected	Total no. metacercariae given	Infection rate* of worms
dog	10- 20	17	61-440	17(100%)	199	292	68.1% (73.8-60.6%)**
	21- 50	11	80-494	11(100%)	275	368	74.7% (79.9-67.9%)
	51-100	13	61-244	13(100%)	672	986	68.1% (71.2-64.7%)
cat	10- 65	13	67-365	13(100%)	275	351	78.3% (85.8-73.3%)

\* : No. worms detected/Total no. metacercariae given.

\*\* : 95% confidence limit.

Table 6. Number of worms and worm-cysts recovered in the treated dogs by autopsy.

No. of dogs	Dose of Bitin	Number of metacercariae given	Days to autopsy from infection	Days to autopsy from beginning of the treatment	Number of worms recovered			Number of worm-cysts		
					survived	dead	total	r-lung	l-lung	total
No. I	1.0g(100mg/kg) X 5	20	266	187	12(60.0%)*	0	12	5(1)**	2(0)**	7(1)**
	1.0g( " ) X 2									
No. II	1.5g(150mg/kg) X 7	100	224	8	11(11.0%)	46	57	12(2)	20(1)	32(3)
	total doses 10.5 grams (daily use)									
No. III	1.5g(150mg/kg) X 10	30	333	16	8(26.6%)	1	9	3(0)	7(5)	10(5)
	total doses 15.0 grams (daily use)									
No. IV	1.5g(150mg/kg) X 6	123	584	235	26(21.1%)	2	28	9(2)	8(1)	17(3)
	1.5g( " ) X 5									
	1.5g( " ) X 20 and									
	1.5g( " ) X 4									
	total doses 52.5 grams (every other day or every two days use)									

( )\* : survival rate (number of worms survived/number of metacercariae given)

\* ( )\*\* : number of worm-cysts with necrotic masses of dead worms.

treatment was attempted on the 43rd day after the first course of treatment. Only 2 doses of Bitin were given, because the dog severely hated taking medicine. E. P. D. decreased again slightly by the administration of Bitin but increased again soon after the second administration of Bitin.

The dog was sacrificed on the 266th day after infection. Pathological and parasitological findings were as shown in Table 6. 5 and 2 worm-cysts were found in the right and left lungs respectively, and 2 worms were living together in each of them except one worm-cyst which contained the necrotic masses of the worms. 12 worms removed from the worm-cysts showed active movement in Tyrode's solution at 37°C and many eggs were found in the uterus cavities of these worms. The survival rate of the worms was 60.0%, which was not significant to that of the control as shown in Table 5. However, all of worm-cysts were localized and perifocal

inflammation were very mild and few *Paragonimus* eggs were found in the surface of pleuras and mediastinum.

#### Dog No. II :

Daily dose of 150 mg/kg of Bitin was given daily for 7 times to the dog No. II from 216 days after infection with 100 metacercariae of *P. westermani*. The dog was died by an accident soon after the 7th administration of Bitin and so autopsied.

E. P. D. showing 63,200-938,600 before treatment began to decrease soon after the 3rd administration and dropped down to 21,000 after the 6th administration of Bitin as shown in Figure 2. Pathological and parasitological findings at autopsy were shown in Table 6. 32 worm-cysts, 12 in the right and 20 in the left lungs, were found. 3 out of them were filled with only necrotic masses of dead worms. 57 worms, 56 worms in the 29 worm-cysts and one

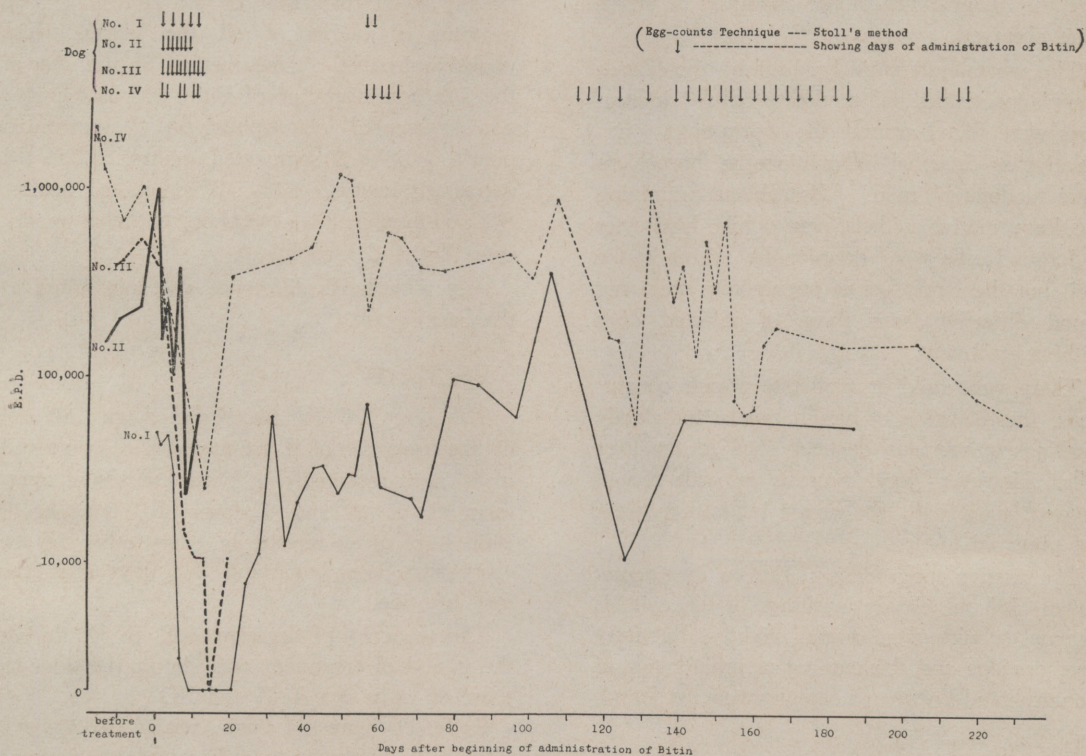


Fig. 2. E.P.D. of Dogs No. I—IV during and after treatment.

worm in pleural cavity, were obtained. 46 out of 57 worms were found to be dead because of severe cloudiness and degenerated atrophy of the bodies. The survival rate of the worms was 11.0% which was significantly lower than that of the control as shown in Table 5. The figures of uterus of these dead worms were all undistinguished and no eggs were found in uterus of them. Only 4 worms out of 11 living worms had a few eggs or the glancing granules of various size, probably vitelline-granules, in uterus.

The inner organs of the worms showing cloudiness or degeneration were microscopically examined. The figures and contours of their uterus were undistinguishable and no eggs were found or many brown granules in irregular size were found in uterus cavities as shown in Plate 1. and 2. (1~5).

Noticeable atrophy of the subcuticular muscle cells and droplets or vascular degenerations in vitelline-glands, especially the resolution or necrosis in the parenchyma cells of ovaries and testes and the disappearance of the endothels of uterus were observed.

The worm-cysts were localized by the fibrous granulation tissues and moderate cellular infiltrations were found around the worm-cysts. Exudative or catarrhal inflammations in bronchiens were moderately mild. Bronchiens containing the desquamative epithels, neutrophils, histiocytes and round cells were occasionally found in the foci, but the large foci of pneumonias were not found differing from those of infected dogs without treatment.

There were mild or moderate passive congestions, the dilatation of lymph canals, and cloudy swellings or vascular degenerations in the liver cells. However, focal necrosis or wide foci of haemorrhages with the severe infiltrations were not observed.

The passive congestions in kidneys were represented, and the cloudy swellings of the epithels of renal tubules were found slightly, but fatty degenerations and thickening or proliferation of glomeruli and Bowman's capsules were not found.

#### Dog No. III :

The dog No. III was infected with 30 metacercariae of *P. westermani*.

150 mg/kg of Bitin were administrated daily for 10 times beginning 317 days after infection. E. P. D. showing 126,000-1,033,500 before treatment decreased gradually with the beginning of treatment, and dropped down to 17,000 after the 7th administration and to 1,440 after the 10th administration of Bitin as shown in Figure 2. The dog was sacrificed on the 7th day after the last administration of Bitin. 10 worm-cysts, 3 in the right and 7 in the left lungs, were found. 5 worm-cysts out of 10 worm-cysts were contained 1-2 worms but the rest of them, 5 worm-cysts, were filled with only the necrotic masses with many eggs of *P. westermani* instead of the living worms. 9 worms, 7 in the worm-cysts and 2 in the pleural cavity, were obtained. 8 worms out of 9 worms were found to be alive and 3 worms out of 8 living worms had a few ova cells or many brown granules in uterus, but the rest of them had empty uterus. The survival rate of the worms was 26.6% which was significantly lower than that of the control. The contours of uterus of all the worms were undistinguishable. Haemogram of the dog at the 7th administration of Bitin was as follows : non segmented neutrophils 0 ; 2 segmented neutro. 6% ; 3 segmented neutro. 22% ; 4 segmented neutro. 24% ; 5 segmented neutro. 8% ; eosinophils 8% ; lymphocytes 24% ; monocytes 8% and basophils 0.

Any remarkable difference was not found in the haemogram.

#### Dog No. IV :

The dog No. IV was infected with 80 and 43 metacercariae of *P. westermani* in an interval of 60 days repeatedly. Four courses of treatment, 6, 5, 20 and 4 doses of 150 mg/kg of Bitin were given repeatedly every other day or every two days beginning 348 days after the first infection.

The tendency of decrease of E. P. D. during the period of treatment was almost the same as those of Dogs No. I, II and III.

E. P. D. increased again within 10-20 days after each treatment as well as the above-mentioned cases. However, E. P. D. decreased

gradually by repeated administration, as shown in Fig. 2.

The dog was sacrificed on the 235th day after the beginning of the first treatment (at the 584th day after the first infection). 9 and 8 worm-cysts were found in the right and left lungs respectively, as shown in Table 6. 2 out of 9 worm-cysts in the right lung and 1 out of 8 worm-cysts in the left lung were filled with the necrotic masses of the dead worms. 28 worms were obtained from the 14 worm-cysts. 26 worms out of 28 worms were alive and they had many eggs in their uterus cavities. The survival rate of the worms was 21.1%. It was interesting that several localized foci found in the lungs were seemed to be the scars of the absorbed worm-cysts judging from the tissue reactions of the fibrous granulations and the existence of many *Paragonimus* eggs.

#### Side effects.

The special attentions were paid to the side-effects during the course of treatments in all cases. Loose stool, diarrhea and anorexia were always found at the first or second administration of Bitin but these symptoms disappeared without any therapy. The loss of body weight was not found during the course of treatment.

#### B. Effects of Bitin on the infected rats with *P. ohirai*.

##### 1. Effects on the adult stage:

The experimental rats and control rats were

all infected with 6 metacercariae of *P. ohirai* at the same time. 5 and 14 rats were given every other day with 4-6 doses of 50 mg/kg and 100 mg/kg of Bitin respectively and were sacrificed during the period from 44 days to 68 days after infection.

The control rats without treatment were also sacrificed at almost the same period. The number of the survival worms recovered from the treated rats and that of the control rats were compared as shown in Table, 7.

The rate of the survival worms described here means the rate of the number of living worms found in the rats belonging to the certain group to the total number of metacercariae given to the rats. The rates of the survival worms of the groups treated with 50 mg/kg and 100 mg/kg were 20.0% and 10.7% respectively. However, that rate of the control group was 66.6%. These rates were significantly lower than that of the control group. The pathological changes of the lungs were compared individually and some of the interesting cases were summarized in Fig. 3. The authors found in other study that the formation of a worm-cyst in the lungs has never been occurred by single worm infection and more than two worms were always found in a worm cyst. Therefore, the worm-cyst containing necrotic masses shown in Fig. 3 contained once more than two worms. E. P. D. of the treated rats decreased rapidly after the 3rd of 4th administration of Bitin, as shown in Figure 4.

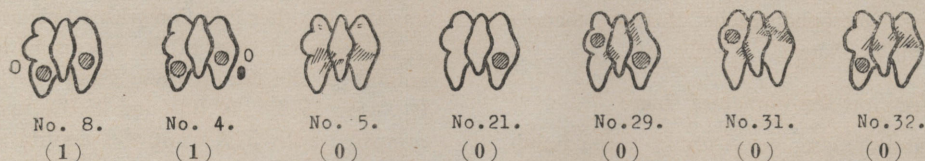


Fig. 3. Pathological changes of lungs of rats treated with Bitin.

- Worm-cyst containing the necrotic masses.
- ▨ fibrous pleurisy.
- survival worm.
- dead worm.
- ( ) showing number of worms survived.



Table 7. Effects of Bitin on adult and immature worms of *P. ohirai* in rats.

Group examined	Dose and method of administration	Time of the beginning of treatment (days before or after infection)	Days from infection to autopsy	No. of the rats used	No. of metacercariae given per rat	Total no. of metacercariae given	Total no. of survival worms	Survival rate*
Adult worms :								
(I)	50 mg/kg X 4-6 every other day	53-68 after infection	68-79	5	6	30	6	20.0% (35.5 - 9.0%)**
(II)	100 mg/kg X 4-6 "	44-68 "	63-76	14	6	84	9	10.7% (18.0 - 5.0%)
Control	— —	—	40-67	6	6	36	24	66.6% (80.0 - 52.0%)
Immature worms :								
(A)	50 mg/kg X 13 every other day	11 after infection	32-38	15	10	150	26	17.3% (23.5 - 13.0%)
(B <sub>1</sub> )	50 mg/kg X 2 (before infect.) " X 4 (after infect.)	4 before infection	26-29	5	10	150	29	58.0% (70.0 - 45.5%)
(B <sub>2</sub> )	50 mg/kg X 2 (before infect.) " X 13 (after infect.)	4 "	26-29	8	10	80	20	25.0% (34.0 - 17.0%)
Control	— —	—	29-32	12	10	120	63	52.5% (60.5 - 44.5%)

\* — no. worms survived/total no. metacercariae given.

\*\* 95% confidence limit.

Pathological changes in the foci of the lungs were very similar as those of the treated dogs with Bitin. Microphotographs of those changes were shown in Plate 2. (6~8).

## 2. Effects on the immature stage.

Each rat belonging to A-group was administrated with 50 mg/kg of Bitin every other day for 13 times beginning 11 days after infection with 10 metacercariae of *P. ohirai*.

Each rat belonging to B<sub>1</sub>-group was administrated every other day with 50 mg/kg of Bitin for 2 times before infection and for 4 times immediately after infection with 10 metacercariae of *P. ohirai*.

Each rat belonging to B<sub>2</sub>-group was admini-

strated every other day with 50 mg/kg of Bitin for 2 times before infection and for 13 times immediately after infection with 10 metacercariae.

The survival rates of the worms found at the autopsy of the rats of these groups, A, B<sub>1</sub> and B<sub>2</sub>, were 17.3%, 58.0% and 25.0% respectively compared with 52.5% of the control group as shown in Table 7. The survival rates of A and B<sub>2</sub>-groups were significantly lower than those of B<sub>1</sub>-group and the control group. It was found that Bitin could be used successfully against the immature worms, when more than 10 doses of Bitin were administrated.

The time of the autopsy after treatment was between 26 and 38 days after infection. The time of autopsy corresponded to the time reach-

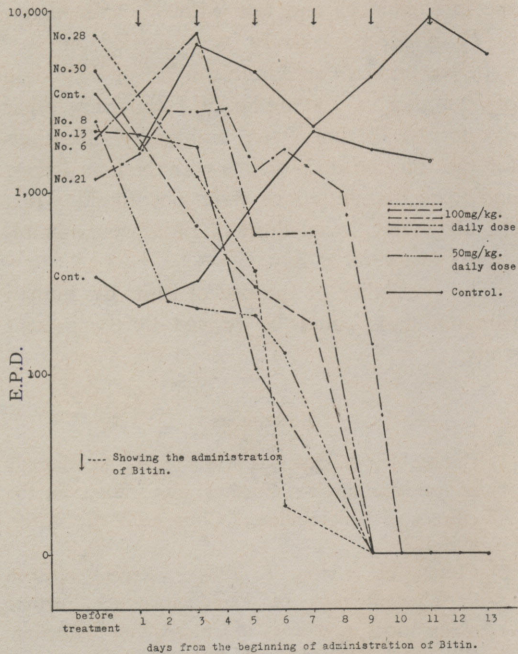


Fig. 4. E. P. D. of the rats treated with Bitin

ing maturity of *P. ohirai* in rat and natural death of the worms usually was not found in this period. Therefore, it may be considered that the survival rates of the worms would be more reliable than those of the adults worms passed for 2 or 3 months after infection. At the autopsy of the treated groups most of the worms found in pleural cavities or liver were still immature. However, most of the worms found in the control group reached maturity and their uterus were filled with many eggs.

Ulcerations and scars caused by penetration of the worms to the lungs or livers of the treated rats were mild in comparison with those of the control group.

### Discussion

S. Yokogawa *et al.* (1937-1941) obtained the excellent results by the use of combined treatment of Emetine and Prontosil. In their experiments they paid special attention to the pathological changes in the lung foci as the criterion of the effect at the experimental chemotherapy of the

dogs infected with *P. westermani* and they also reported that it was a question whether E. P. D. during the period of treatment could justify the effect of the drug or not. Afterwards many studies on the treatment for animal or human paragonimiasis were reported by Iwata (1942), Mori (1948), Komiya *et al.* (1952), Tanaka (1958), Suguro (1959) and others.

Recently Suguro (1959) reported that the effects of the drugs on the infected dogs with *P. westermani* would be able to be compared by E. P. D., when the examinations of E. P. D. carried out continuously for a long period. He tried various drugs, such as Emetine hydrochloride with sulfonamide, Chloroquine and Atabrine, for the chemotherapy of the infected dogs with *P. westermani* and found that none of them could not diminish the eggs in feces unless the treatment was continued at least for more than a month. Komiya *et al.* (1952) reported that the treatment with the combined method of Emetine hydrochloride and sulfonamide for 10 days was not enough for human paragonimiasis.

In the present study Bitin seemed to be quite effective on 3 cases except 1 case, No. 1 dog. It has never been experienced by any other drugs ever used that E. P. D. decreased so quickly after 2 or 3 administrations as Bitin. It was very interesting that the several localized foci suggestive of scars of the absorbed worm-cysts were found in the case of No. IV dog. This fact may indicate the strong effect of Bitin. The rats infected with *P. ohirai* have never been used for the experimental chemotherapy of paragonimiasis. It was proved that the infected rats with *P. ohirai* could be ideally used for the experimental chemotherapy by the present study.

This fact would be quite advantageous for the preliminary test of experimental chemotherapy or the screening of the effective drugs on paragonimiasis because paragonimiasis of rats can be easily raised at the laboratory.

Bitin was also quite effective against the immature stage of *P. ohirai* as well as the adult stage in this experiment. The effectiveness of Emetine hydrochloride against the immature stage of *P. westermani* has never been investigated *in vivo*.

The further investigation is needed to compare the resistances of the various stages of the worms of *P. westermani* and *P. ohirai* against the drugs.

As the method of evaluation of the effect of drugs, the comparison of the survival rates of the worms were seemed to be quite useful especially for immature worms.

In the present study the mechanisms of the effect of Bitin against the worms *in vitro* or *in vivo* were not still clear.

However, it was proved that the administration of 50 mg/kg or 100 mg/kg of Bitin every other day for 10-15 times was seemed to be more effective and less toxic for the infected mice and rats than Emetine hydrochloride.

The side-effects as described above were found at the first period of treatment with Bitin, but disappeared naturally without treatment. The decrease of the body weight of the treated animals was not found and the haemogram of the treated dogs with Bitin were almost the same as those of normal untreated dogs.

The most of the survival worms removed from the treated dogs or rats had not eggs in uterus, and degenerated changes in uterus, ovaries, testes and vitelline glands were quite remarkable, but the some of those worms seemed to be regenerate again their vitalities within 10-20 days after treatment when the doses of Bitin were insufficient. The possibility of the regeneration of the survival worms will be really understood not only from the above-described results but from the facts that the most of the relapses of human paragonimiasis has been seen within one month after the therapy, as reported by Komiya *et al.* (1952).

It was interesting that the temporal increasing of E. P. D. after the several doses of Emetine hydrochloride or Chloroquine were not observed by the administration of Bitin.

### Summary

From the results of the examination of the direct effect of Bitin on excysted metacercariae of *P. westermani in vitro*, it was found that Bitin had eminent effect for the lung flukes.

The experimental chemotherapy with Bitin for the infected dogs and rats with *P. westermani* or *P. ohirai* respectively, were carried out.

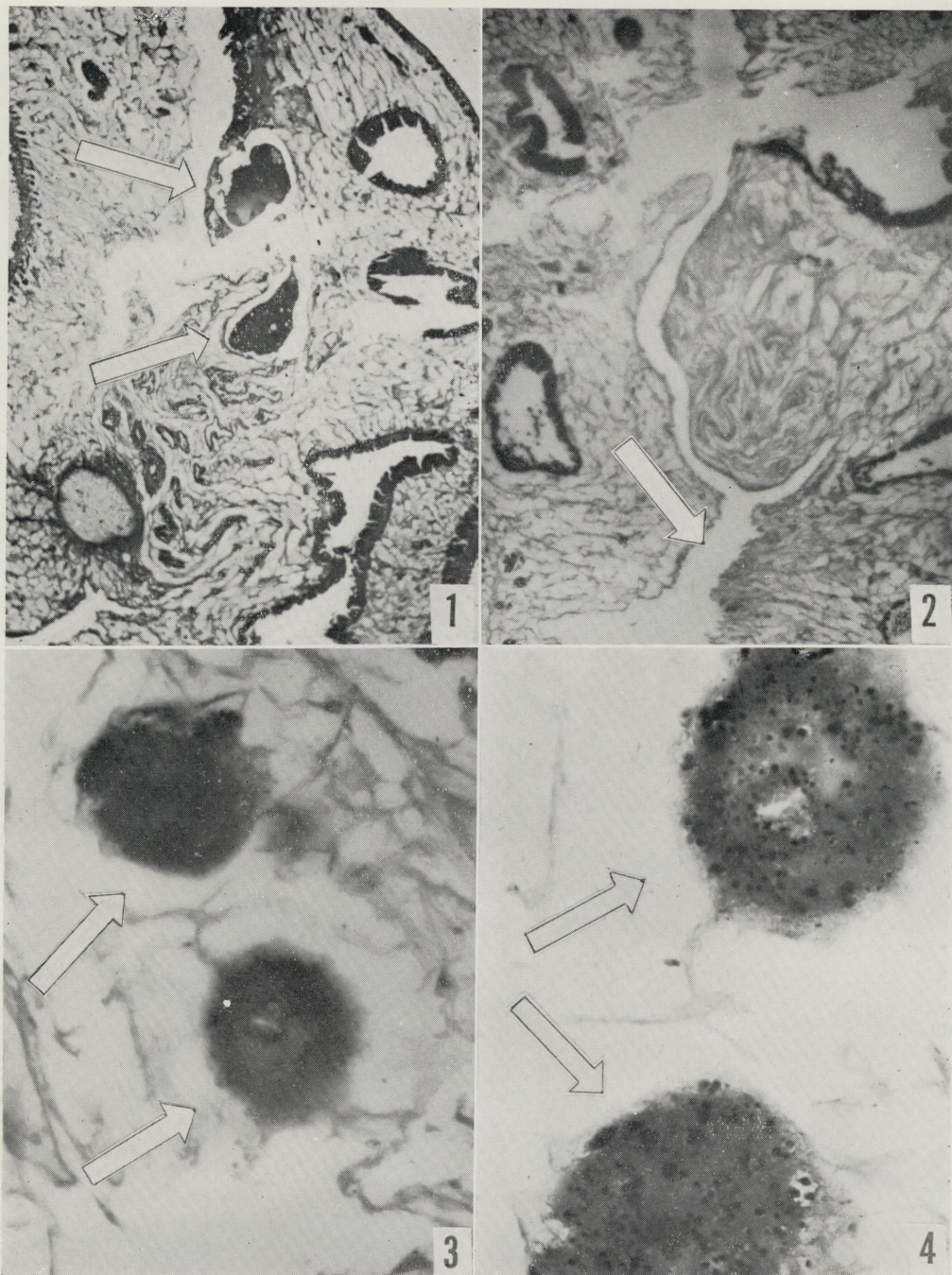
It was proved that administration of daily dose of 50 mg/kg or 100 mg/kg of Bitin every other day for 10-15 times were eminently efficacious for the infected dogs or rats with the lung flukes, and toxic manifestations were not found when the above-mentioned doses of Bitin were administered every other day.

The possibility of the use of Bitin for human paragonimiasis could be proved by the present study.

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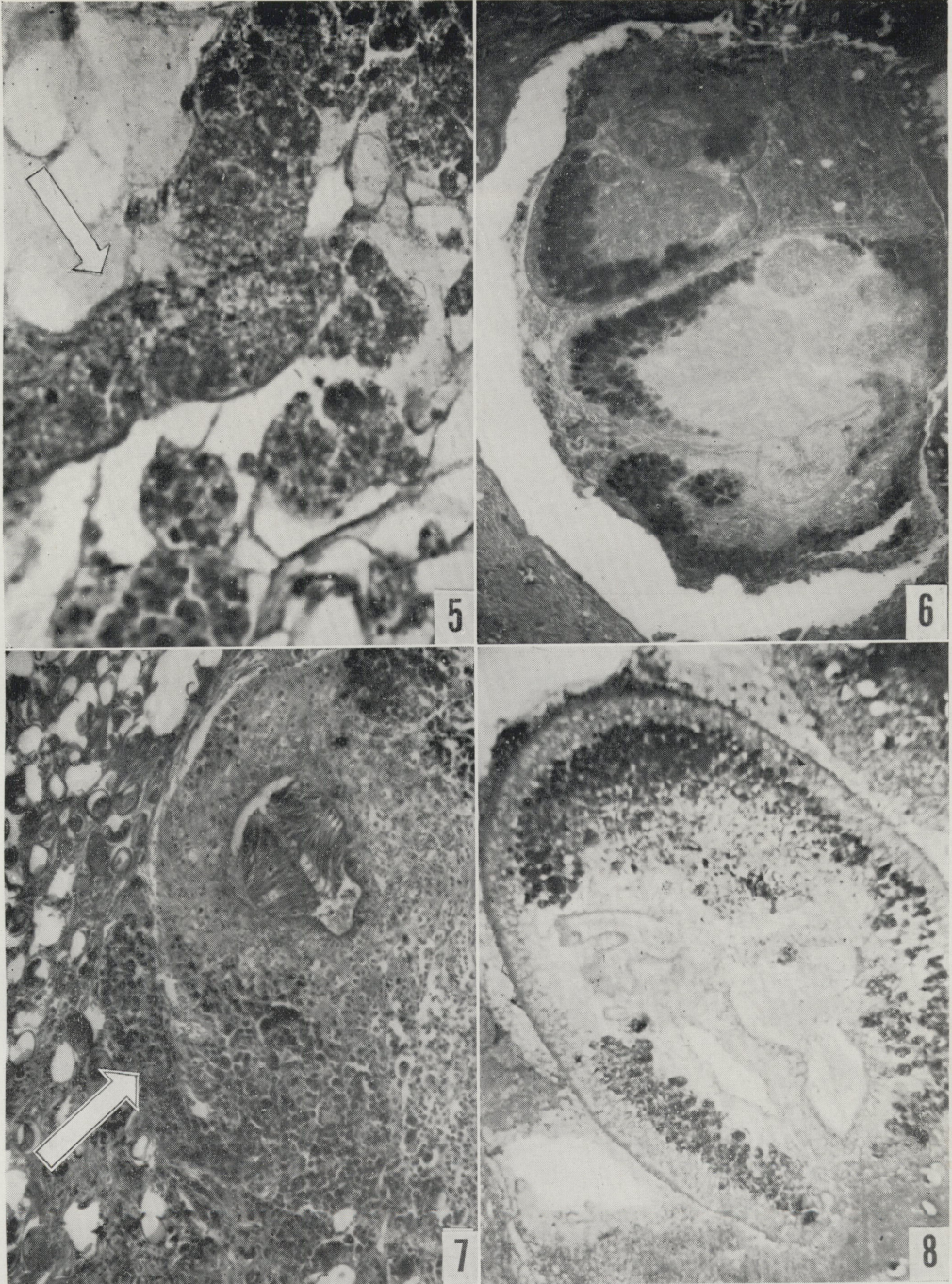
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1. Section of the worm removed from the lung of Dog, No. II.  
Arrows indicate the advanced degeneration of ovary of the worm.
2. Same as the above.  
No eggs are found in the uterus cavity and the endothelium are irregular or disappeared.
3. Section of the worm removed from the lung of Dog, No. III.  
Ovary showing the advanced degeneration and disappearance of the parenchyma cells.
4. Same as the above.  
Testis showing pycnosis or karyolysis of the parenchyma cells.

## Plate 2.



5. Same as the above.  
Vitelline-glands showing hyaline droplet degeneration.
6. Section of the worm-cyst removed from the lungs of rat No. 6.  
2 worms showing necrobiosis in the worm-cyst.
7. Section of the worm-cyst removed from the lung of rat No. 21.  
Arrow indicates accumulation of yellow pigment-granules.
8. Section of the worm removed from the lung of rat No. 13.  
No eggs are found in the cavity of uterus of the worm.

肺吸虫症の化学療法に関する研究  
 (I) Bithional (Bitin) による動物肺吸虫症の治療成績

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1956年以來、著者等はウェステルマン肺吸虫脱囊幼虫の体外飼育法により各種薬剤の本種幼虫に対する直接効果を検索して来たが、今回 Bithional (Bitin) がこれまで知られた Emetine, Atabrine, Chloroquine (Resochin) 等と比べて数倍の強い殺虫効果のある事を見出した。そこで本剤による動物肺吸虫症の治療実験を企図しその使用量を決定するために Bitin のマウス及びラットに対する中毒試験を行つた結果 100 mg~150 mg/kg を隔日に経口的に継続しうる事が明らかにされた。そこでウェステルマン肺吸虫犬4頭及び今回始めて大平肺吸虫メタセルカリアを6~10ヶ投与した感染ラットについて治療を行つた。之等治療効果の判定には治療前、治療期間中及び治療後における糞便内排卵数 (E. P. P.) の消長を犬については全例、ラットではその一部について対照群と比較観察すると共に一定期間の治療終了後剖検によつて肺臓病変、虫体生存率 (投与メタセルカリア数に対する生存虫体数の百分比) 並びに生殖器官を中心とした虫体の形態学的所見を対照群のそれらと比較観察した。何れの犬においても Bitin 100 mg~150 mg/kg 隔日、3~4回投与により E. P. D. は急激に減少したが治療が不十分であるときは大凡3週日目より E. P. D. は再び増加し、更に治療を反覆することによつて次第に減少した。剖検所見では虫囊の一部は壊死虫体を包蔵し、生存虫体の多くは変性過程にあり、子宮、卵巣、睪丸等の著明な変性が見とめられた。大平肺吸虫成虫に対してもウェステルマン肺吸虫の場合と略々同様で、50 mg/kg 及び 100 mg/kg 隔日4~6回投与で虫体生存率は20.0%及び10.7%を示し対照群の66.6%と比べて著しく低かつた。猶本種幼虫期のものに対しても同様試みた結果は、Bitin を一定期間継続する事によつてその効果が期待されるものと推察された。こゝに Bitin がウェステルマン肺吸虫及び大平肺吸虫に対して共に有効である事が明らかにされ、且動物肺吸虫症の治療実験には大平肺吸虫感染ラットを用いる事が極めて好都合である事が同時に明らかにされた。加えて本剤の人体肺吸虫症の臨床的応用への可能性を明らかにした。