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STUDIES ON EFFECTS OF DRUGS UPON PROTOSCOLECES OF *ECHINOCOCCUS GRANULOSUS* IN VITRO

I SCOLICIDAL EFFECT OF SALICYLANILIDE AND BISPHENOL DERIVATIVES AGAINST *ECHINOCOCCUS GRANULOSUS* IN VITRO*1

Tsukasa SAKAMOTO and Michael A. GEMMELL*2

Department of Parasitology
Faculty of Veterinary Medicine
Hokkaido University, Sapporo, Japan

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The protoscoleces of *Echinococcus granulosus* (BATSCH, 1786) were incubated for ten days in media to which a range of salicylanilide- and bisphenol-derivatives and other compounds were added at the rates of 1, 10 and 100 γ per ml. The main culture medium was 20% inactivated calf serum with a 1:1 mixture of medium 199 and HANKS' solution containing 0.5% lactalbumin hydrolysate and 0.1% yeast extract. The survival rates of the treated and control protoscoleces were compared.

Generally, halogenated salicylanilide derivatives showed the highest scolicidal effect and halogenated derivatives of diphenyl sulfide and diphenylmethane ranked next in lethal effect. The intensity of the scolicidal action of salicylanilide derivatives increased with the addition of some halogen atoms. Bisphenol derivatives also showed an increase in scolicidal activity associated with the number of halogen atoms.

Descriptions were made of the activity of two types of salicylanilide derivatives and three bisphenol compounds suspended in media with propylene glycol or sodium carboxymethyl cellulose. The drugs in the former manifested a stronger scolicidal effect than in the latter category.

INTRODUCTION

Two principal approaches have been used to screen chemicals that kill or inhibit growth of the larval tissue of *Echinococcus*. One of them is pharmaco-therapeutic experiment using mice artificially infected with *Echinococcus*. LUBINSKY (1969) and LUBINSKY et al. (1971) utilized this pharmaco-therapeutic approach to test drugs against secondary multilocular echinococcosis in mice. The other is

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*2 Hydatid Research Unit, Ministry of Agriculture and Fisheries of New Zealand

TABLE 1 *Lethal times for 10, 25, 50, 75 and 90 per cent of protoscoleces in the medium*[*1] *containing drugs at three concentrations*

NO.	DRUG	LT_{50} DOSE/ml			LT_{90} DOSE/ml		
		1 γ	10 γ	100 γ	1 γ	10 γ	100 γ
	DIPHENYL SULFIDE DERIVATIVE	day	day	day	day	day	day
1	2, 2′-Thiobis (4-chlorophenol)	—	4.49	### [*3]	—	7.28	###
2	2, 2′-Thiobis (4-bromophenol)	6.17	4.24	###	12.92	9.69	###
3	2, 2′-Thiobis (4-fluorophenol)	—	(8.36/10)[*2]	###	44.08	26.57	###
4	2, 2′-Thiobis (4, 6-dichlorophenol)	3.23	###	###	5.19	###	###
5	2, 2′-Thiobis (4, 6-dibromophenol)	(9.05/25)	4.34	###	14.02	—	###
6	4, 4′-Thiobis (2, 6-dichlorophenol)	—	(6.96/10)	3.99	135.55	22.04	6.92
7	2, 2′-Thiobis (4-chloro-6-nitrophenol)	(6.14/10)	###	###	23.48	###	###
8	2, 2′-Thiobis (4-bromo-6-nitrophenol)	—	3.27	###	61.34	6.53	###
9	Thiobis (2-acetoxy-3-nitro-5-chlorobenzene)	9.18	3.47	###	12.17	6.06	###
10	Thiobis (2-methoxy-3-nitro-5-chlorobenzene)	—	6.47	3.38	65.95	9.13	6.02
11	Thiobis (2-acetoxy-3, 5-dichlorobenzene)	—	3.81	1.08	33.89	6.09	4.33
12	2, 2′-Thibis (4-chloro-6-bromophenol)	(7.76/10)	3.98	###	—	—	###
13	Thiobis (2-carboxymethoxy-3, 5-dichlorobenzene)	(7.58/10)	9.73	###	23.99	—	###
14	2, 2′-Thiois (3, 4, 6-trichlorophenol)	3.42	(2.08/75)	###	5.33	3.69	###
15	Thiobis (2-acetoxy-3, 5, 6-trichlorobenzene)	(7.05/25)	4.32	2.84	14.92	—	6.74
16	Disodiumthiobis (3, 4, 6-trichlorophenolate)	7.58	4.11	###	—	9.96	###
17	Disodiumthiobis (4-chloro-6-nitrophenolate)	—	(8.35/10)	###	—	22.24	###
18	Tripiperazine tetrabithionol	4.84	3.74	###	8.40	5.56	###
	DIPHENYL SULFOXIDE DERIVATIVE						
19	2, 2′-Sulfinylbis (4, 6-dichlorophenol)	(8.82/25)	2.50	###	17.92	4.74	###
20	2, 2′-Sulfinylbis (4-chloro-6-nitrophenol)	—	(5.86/10)	###	—	23.89	###
21	8-Hydroxy-quinoline-bithionol sulfoxide	(7.23/10)	4.56	###	27.46	8.03	###
22	Diethylcarbamazine-bithionol sulfoxide	(10.25/10)	8.99	(1.88/75)	—	13.02	3.68
	DIPHENYL SULFONE DERIVATIVE						
23	2, 2′-Sulfonylbis (4-chloro-6-nitrophenol)	(8.49/10)	3.51	###	47.22	5.81	###
24	Sulfonylbis (2, 3, 6-trichlorobenzene)	—	—	(9.73/10)	68.71	37.77	29.74
	DIPHENYL DISULFIDE DERIVERTIVE						
25	2, 2′-Dithiobis (4-chlorophenol)	—	(9.76/10)	###	54.07	41.48	###
26	2, 2′-Dithiobis (4, 6-dichlorophenol)	—	4.08	###	47.10	6.77	###
27	2, 2′-Dithiobis (4-chloro-6-nitrophenol)	(4.03/10)	2.46	###	52.55	4.72	###
	DIPHENYL TRISULFIDE DERIVATIVE						
28	2, 2′-Trithiobis (4-chloro-6-nitrophenol)	—	(2.46/75)	###	89.11	3.90	###
	DIPHENYL METHANE DERIVATIVE						
29	2, 2′-Methylenebis (4-chlorophenol)	7.47	3.31	###	11.27	5.41	###
30	2, 2′-Methylenebis (4-nitrophenol)	7.64	2.97	###	14.76	5.75	###
31	2, 2′-Methylenebis (4-methylphenol)	(9.05/10)	(8.59/25)	9.01	22.16	13.82	—
32	2, 2′-Methylenebis (4-chloro-6-aminophenol)	—	—	###	—	53.55	###
33	2, 2′-Methylenebis (4-chloro-6-nitrophenol)	7.20	3.09	###	10.19	5.72	###
34	2, 2′-Methylenebis (4-chloro-6-bromophenol)	6.84	3.92	###	12.42	7.03	###
35	2, 2′-Methylenebis (4-nitro-6-bromophenol)	5.91	3.75	###	10.98	6.81	###

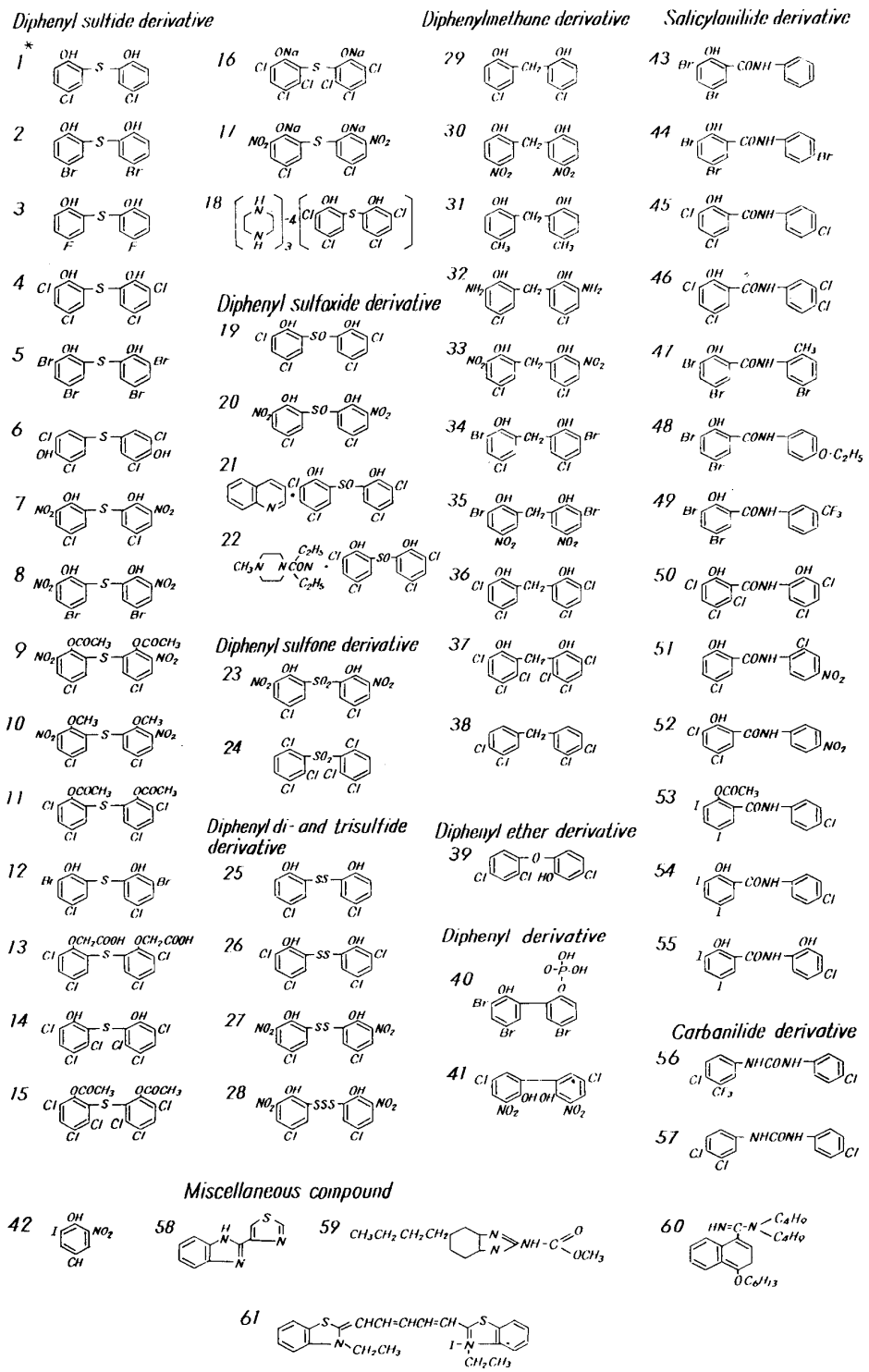
NO.	DRUG	LT_{50} DOSE/ml			LT_{90} DOSE/ml		
		1γ	10γ	100γ	1γ	10γ	100γ
		day	day	day	day	day	day
36	2, 2′-Methylenebis (4, 6-dichlorophenol)	4.95	2.91	###	8.40	4.71	###
37	2, 2′-Methylenebis (3, 4, 6-trichlorophenol)	3.77	2.50	###	6.87	5.08	2.34
38	Methylenebis (3, 4-dichlorobenzene)	—	(8.73/25)	4.49	32.53	14.73	—
	DIPHENYL ETHER DERIVATIVE						
39	2, 4, 4′-Trichloro-2′-hydroxydiphenyl ether	7.65	3.33	###	9.97	5.58	###
	DIPHENYL DERIVATIVE						
40	4, 4′, 6, 6′-Tetrabromo-2, 2′-biphenyldiolmono (dihydrogenphosphate)	9.25	4.39	###	14.40	—	###
41	5, 5′-Dichloro-3, 3′-dinitro-2, 2′-biphenyldiol	(3.95/75)	(2.32/75)	###	7.25	3.85	###
	PHENOL DERIVATIVE						
42	4-Cyano-2-iodo-6-nitrophenol	(9.75/10)	9.25	###	24.62	11.08	###
	SALICYLANILIDE DERIVATIVE						
43	3, 5-Dibromosalicylanilide	7.52	(8.64/75)	###	20.81	13.31	###
44	3, 5, 4′-Tribromosalicylanilide	7.48	###	###	12.55	2.98	###
45	3, 5, 4′-Trichlorosalicylanilide	5.36	###	###	14.01	###	###
46	3, 5, 3′, 4′-Tetrachlorosalicylanilide	(6.42/75)	###	###	11.68	###	###
47	3, 5, 5′-Tribromosalicyl-o-toluidide	###	###	###	—	###	###
48	3, 5-Dibromo-4′-ethoxysalicylanilide	(6.30/25)	1.90	###	23.82	—	###
49	3, 5-Dibromo-3′-trifluoromethylsalicylanilide	###	###	###	3.99	###	###
50	3, 3′, 5, 5′, 6-Pentachloro-2, 2′-dihydroxybenzanilide	3.88	2.44	###	—	5.21	###
51	2′, 5-Dichloro-4′-nitrosalicylanilide	###	###	###	###	###	###
52	3, 5-Dichloro-4′-nitrosalicylanilide	2.69	###	###	9.31	###	###
53	4′-Chloro-3, 5-diiodosalicylanilide acetate ester	—	(8.83/25)	4.41	83.85	13.53	7.15
54	4′-Chloro-3, 5-diiodosalicylanilide	—	6.84	###	29.75	9.00	###
55	4′-Chloro-2′-hydroxy-3, 5-diiodosalicylanilide	(9.08/10)	5.89	###	17.33	8.71	###
	CARBANILIDE DERIVATIVE						
56	3-Trifluoromethyl-4, 4′-dichlorocarbanilide	1.84	###	###	4.84	###	###
57	3, 4, 4′-Trichlorocarbanilide	3.06	(3.13/75)	###	5.17	4.35	###
	BENZIMIDAZOLE DERIVATIVE						
58	Thiabendazole hydrochloride	(9.70/25)	(7.61/25)	5.58	15.05	13.33	8.86
59	Parbendazole	(9.20/10)	(8.02/10)	8.92	—	—	—
	BUNAMIDINE DERIVATIVE						
60	Bunamidine hydrochloride	3.48	###	###	5.96	###	###
	CYANINE DYE						
61	Dithiazanine iodide	(7.53/10)	4.38	3.52	23.46	7.90	6.33

*1 The medium contains propylene glycol at a concentration of 0.5 per cent with drug.

*2 Numbers parenthesized do not belong to LT_{50} but LT_{10}, LT_{25} and LT_{75} respectively, as shown by denominators.

*3 In cases where quadratic equation is unable to be computed, the lethal times while survival percentages become less than 50 or 10 per cent, are expressed by 5, 4, 3, 2 and 1 plus signs in order of time on the 2nd, 4th, 6th, 8th and 10th days of incubation, respectively. The things which the survival percentages are more than 50 or 10 per cent by the 10th day are indicated by dash.

FIGURE 1 *Formulae of drugs tested*



* The numbers of formulae shown in this figure are same them in table 2.

research on the in vitro scolicidal properties of drugs. MEYMERIAN et al. (1963) and FRAYHA et al. (1971) determined the in vitro scolicidal activity of drugs by tests for the mobility and staining properties of protoscoleces of *E. granulosus*. SCHWABE et al. (1963) and LUKASHENKO et al. (1970, 1971) and KOVALENKO (1971) tested the inhibitory effects of chemical compounds on the respiratory rate of protoscoleces of *E. granulosus* and *E. multilocularis*, respectively.

SAKAMOTO et al. (1965) and SAKAMOTO (1973) assessed the in vitro scolicidal activity of some drugs against protoscoleces of *E. multilocularis* on the basis of cytopathological examination. The present paper extends previous studies and reports the results of tests on the in vitro scolicidal activity of salicylanilide derivatives, bisphenol compounds and others against protoscoleces of *E. granulosus*.

MATERIALS AND METHODS

Livers with echinococcal cysts were collected from sheep naturally infected with *Echinococcus granulosus* (BATSCH, 1786) at an abattoir in the South Island of New Zealand. Echinococcal cysts were separated aseptically from the connective tissue of the host. Hydatid sand was obtained from the cyst with a syringe. The protoscoleces were separated mechanically from the brood capsule following trypsinization. The viable protoscoleces were thoroughly washed with HANKS' solution. The culture medium used was 20% inactivated calf serum with a 1 : 1 mixture of MORGAN, MORTOM & PARKER's 199 and HANKS' solution containing 0.5% lactalbumin hydrolysate and 0.1% yeast extract. To this medium were added 100 units of penicillin G and 100 γ of streptomycin sulfate per ml. About 15,000 protoscoleces in 10 ml of medium at 37°C were incubated in each culture tube. The medium (pH 7.4) was changed every other day.

The 61 drugs tested included 13 types of salicylanilide derivatives, 41 bisphenol derivatives, a phenol derivative, 2 carbanilide derivatives, 2 benzimidazole derivatives, a bunamidine derivative and a cyanine dye. The bisphenol derivatives consisted of 18 diphenyl sulfides, 4 diphenyl sulfoxides, 2 diphenyl sulfones, 3 diphenyl disulfides, a diphenyl trisulfide, 10 diphenyl methanes and 3 other diphenyl compounds. The identity of each drug is shown in table 1 and figure 1.

The compounds were dissolved or suspended in propylene glycol at rates of 0.2, 2 and 20 mg per ml. The solution of suspension was added to the medium at a rate of 0.005 ml per ml to provide 1, 10 and 100 γ/ml concentrations of drugs. Triplicate samples of each drug at each dose rate in the medium were tested for scolicidal activity. Morphological changes were assessed every time when the media were changed. These observations were continued for up to ten days using normal and phase-contrast microscopes and supravital staining with neutral red and Janus green.

RESULTS

The values of the mean and standard error of the survival rates of the controls in all experiments were estimated from the two media containing propylene glycol or sodium carboxymethyl cellulose only (tab. 2).

TABLE 2 *Rates of survival protoscoleces in two media containing propylene glycol and sodium carboxymethyl cellulose respectively*

ADDITIVE IN MEDIUM	DURATION OF INCUBATION				
	2 days	4 days	6 days	8 days	10 days
	%	%	%	%	%
0.5% propylene glycol	99.92 ± 0.01*	99.78 ± 0.02	99.61 ± 0.03	99.24 ± 0.04	98.73 ± 0.07
0.3% sodium carboxymethyl cellulose	99.95 ± 0.03	99.86 ± 0.07	99.60 ± 0.05	99.36 ± 0.02	99.08 ± 0.12

* Mean ± standard error

In the preliminary assessments, the concentration of drug in each medium for mortalities of 50 and 90 per cent of protoscoleces (LC_{50} & LC_{90}) were estimated for five drugs by means of the logarithmic-probit graphic method[1,2] (tab. 3). These values obtained, however, were not necessarily ideal as indices measuring the scolicidal potency of drugs because they sometimes varied wide limits over time.

The longevity of treated protoscoleces was transformed into survival percentages with respect to their controls. The regression curves (time-mortality curves) were computed from those survival percentages over time. These curves were expressed by quadratic equations in terms of survival percentage (y) and exposure time (x) (fig. 2). The length of exposure time for the 50 and 90 per cent mortality rates (LT_{50} & LT_{90} for protoscoleces) was estimated from the quadratic equations of the regression curves. In cases where the survival percentages were too high or low to estimate the LT_{50} and LT_{90}, estimates of the LT_{10}, LT_{25} or LT_{75} were used as indices of scolicidal potency. In cases where extremely high or low survival percentages occurred and these estimates could not be used, the lethal times while survival percentages become less than 50 or 10 per cent, were indicated the number of plus signs; 5, 4, 3, 2 and 1 plus signs in order of time on the 2nd, 4th, 6th, 8th and 10th days of incubation, respectively. In cases where the survival percentages were more than 50 or 10 per cent, these were expressed by dash sign (tab. 1).

Generally speaking, the derivatives of halogenated salicylanilide revealed the highest scolicidal effect, and halogenated bisphenol derivatives were ranked next to them. Bunamidine hydrochloride also revealed a strong scolicidal effect.

The relation between the chemical structure and the in vitro scolicidal effects

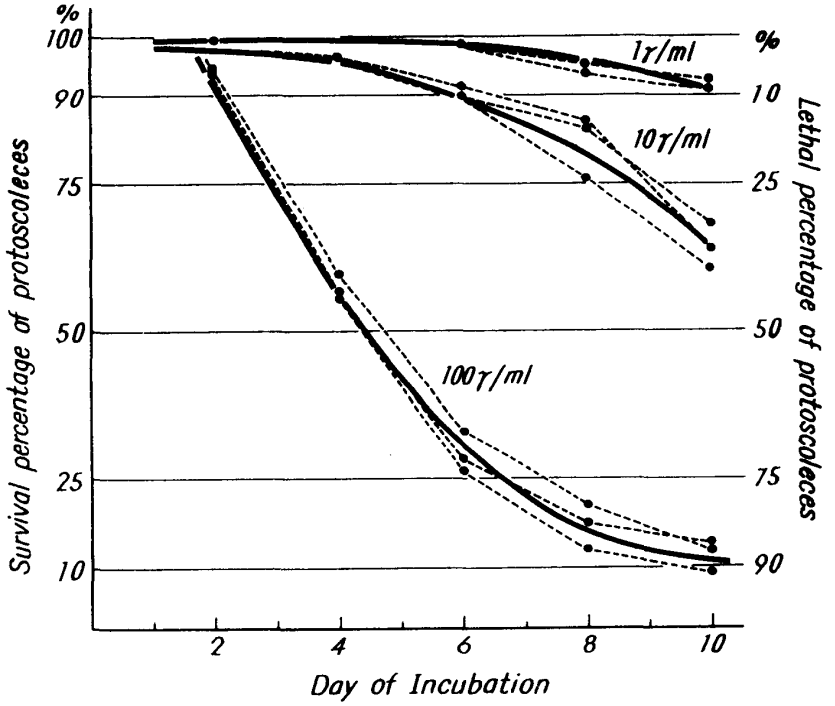
TABLE 3 *LC_{50} and LC_{90} of drugs for protoscoleces in two media containing propylene glycol and sodium carboxymethyl cellulose respectively*

DRUG	LC_{50} FOR PROTOSCOLECES					LC_{90} FOR PROTOSCOLECES				
	DURATION OF INCUBATION					DURATION OF INCUBATION				
	2 days	4 days	6 days	8 days	10 days	2 days	4 days	6 days	8 days	10 days
	γ/ml	γ/ml	γ/ml	γ/ml	γ/ml	γ/ml	γ/ml	γ/ml	γ/ml	γ/ml
2, 2′-Sulfinylbis-(4, 6-dichlorophenol)	1.3×10 (6.3×10^4)*1	3.2 (1.4×10)	2.2 (4.5)	1.8 (2.3)	1.4 —*2	5.0×10 (6.3×10^6)*1	7.9 (5.0×10)	5.6 (1.0×10)	4.0 (4.0)	3.2 (<3.9)
2, 2′-Methylenebis-(4-chloro-6-nitrophenol)	2.2×10^2 (2.2×10^3)	2.8 (3.4)	1.5 (0.4)	1.0 (0.4)	— —	1.3×10^4 (6.3×10^5)	2.2×10 (3.2×10)	1.2×10 (5.0)	2.5 (<1.0)	1.0 —
2, 2′-Methylenebis-(3, 4, 6-trichlorophenol)	4.0 (2.2×10)	0.1 (0.15)	0.08 (0.1)	— 0.1	— —	7.4×10 (4.0×10^2)	1.3×10 (4.0×10)	1.3 (5.0)	0.8 (0.8)	0.8 (0.8)
3, 5, 3′, 4′-Tetrachloro-salicylanilide	0.5 (2.8)	— (0.5)	— —	— —	— —	3.2 (4.0×10)	1.8 (9.7)	1.4 (9.7)	1.2 —	<1.0 —
2′, 5-Dichloro-4′-nitrosalicylanilide	<1.0 (3.2)	— (<1.0)	— —	— —	— —	<1.0 (1.4×10)	— (<1.0)	— —	— —	— —

*1 Numbers parenthesized show the lethal concentrations im medium with sodium carboxymethyl cellulose.

*2 In cases where the lethal concentrations were unable to be estimated, they are expressed by dash.

FIGURE 2 *Regression lines for survival percentages of protoscoleces in the medium* containing methylenebis (3, 4-dichlorobenzene) at three concentrations*



Regression line: Bold solid line
Experimental curve: Fine dotted line
Dose: Quadratic equation
1 γ/ml: $y = -0.091x^2 + 0.226x + 99.067$
10 γ/ml: $y = -0.586x^2 + 2.903x + 94.357$
100 γ/ml: $y = 1.419x^2 - 27.424x + 144.553$

* The medium contains propylene glycol at a concentration of 0.5 per cent.

was investigated with those salicylanilide- and bisphenol-derivatives that gave the most conspicuous scolicidal activity. The scolicidal effect of 3, 5-dibromosalicylanilide and 3, 5-dibromo-4′-ethoxysalicylanilide was weaker than that of 3, 5, 4′-tribromosalicylanilide, 3, 5-dibromo-3′-trifluoromethylsalicylanilide and 3, 5, 5′-tribromosalicyl-o-toluidide. 2′, 5-dichloro-4′-nitrosalicylanilide (=niclosamide) revealed the most effective activity of the salicylanilide derivatives tested in the present experiment. In comparison between three types of salicylanilide derivatives, 3, 5, 4′-trichlorosalicylanilide showed a stronger scolicidal effect than 3, 5, 4′-tribromosalicylanilide, and 4′-chloro-3, 5-diiodosalicylanilide gave the weaker effect. 3, 5, 4′-trichlorosalicylanilide was weaker in effect than 3, 5, 3′, 4′-tetrachlorosalicylanilide and 3, 5-dichloro-4′-nitrosalicylanilide. The in vitro scolicidal effect of 4′-chloro-3, 5-diiodosalicylanilide was stronger than its acetate ester, Clioxanide, but weaker than 4′-chloro-

2′-hydroxy-3, 5-diiodosalicylanilide. The in vitro effect of 3, 3′, 5, 5′, 6-pentachloro-2, 2′-dihydroxy-benzanilide (=Oxyclozanide) was lower than that of 3, 5, 3′, 4′-tetrachlorosalicylanilide.

Of the bisphenol derivatives, diphenyl sulfide- and diphenylmethane-derivatives showed some lethal effect. Of derivatives of diphenyl sulfide, 2, 2′-thiobis (3, 4, 6-trichlorophenol) and 2, 2′-thiobis (3, 4, 6-trichlorophenol) and 2, 2′-thiobis (4, 6-dichlorophenol) (=bithionol) and tripiperazine tetrabithionol showed a more intense effect than 2, 2′-thiobis (4-chlorophenol). 2, 2′-thiobis (4-chloro-6-nitrophenol) a derivative of diphenyl sulfide and 2, 2′-methylenebis (4-chloro-6-nitrophenol) a derivative of diphenylmethane were much higher in scolicidal effect than 2, 2′-thiobis (4-chlorophenol) and 2, 2′-methylenebis (4-chlorophenol) (=dichlorophen). The scolicidal action of 4, 4′-thiobis (2, 6-dichlorophen) was much weaker than 2, 2′-thiobis (4, 6-dichlorophenol). Thiobis (2-acetoxy-3, 5-dichlorobenzene) and thiobis (2-carboxymethyl-3, 5-dichlorobenzene), thiobis (2-acetoxy-3-nitro-5-chlorobenzene) and thiobis (2-methoxy-3-nitro-5-chlorobenzene), and thiobis (2-acetoxy-3, 5, 6-trichlorobenzene) were weaker in scolicidal effect than the parent bisphenols. The scolicidal effect of tripiperazine tetrabithionol, 8-hydroxy-quinoline-bithionol sulfoxide and diethylcarbamazine-bithionol sulfoxide was much weaker than that of bithionol or bithionol sulfoxide. The soluble salts, disodiumthiobis (3, 4, 6-trichlorophenolate) and disodiumthiobis (4-chloro-6-nitrophenolate) revealed a weaker effect than that of the original bisphenols. Sulfonylbis (2, 3, 6-trichlorobenzene) was ineffective against protoscoleces. 2, 2′-sulfonylbis (4-chloro-6-nitrophenol), 2, 2′-sulfonylbis (4-chloro-6-nitrophenol), 2, 2′-dithiobis (4-chloro-6-nitrophenol) and 2, 2′-trithiobis (4-chloro-6-nitrophenol), which were substituted with sulfoxide, sulfone, disulfide and trisulfide in place of the single sulphur linkage of 2, 2′-thiobis (4-chloro-6-nitrophenol) respectively, showed a conspicuous decline in scolicidal effect. 2, 2′-sulfinylbis (4, 6-dichlorophenol) and 2, 2′-dithiobis (4, 6-dichlorophenol) showed a lower scolicidal activity than bithionol.

Of diphenylmethane derivatives, 2, 2′-methylenebis (3, 4, 6-trichlorophenol) (=hexachlorophen) gave the highest scolicidal effect. In contrast, methylenebis (3, 4-dichlorobenzene) and 2, 2′-methylenebis (4-methylphenol) were ineffective against protoscoleces. Of the diphenylmethane derivatives, the order of decreasing activity was 2, 2′-methylenebis (4-chlorophenol), 2, 2′-methylenebis (4-nitrophenol) and 2, 2′-methylenebis (4-methylphenol). Of four diphenylmethanes, the scolicidal effect decreased gradually in the order 2, 2′-methylenebis (3, 6-dichlorophenol), 2, 2′-methylenebis (4-chloro-6-nitrophenol), 2, 2′-methyenebis (4-chloro-6-bromophenol) and 2, 2′-methylenebis (4-chloro-6-aminophenol). 5, 5′-dichloro-3, 3′-dinitro-2, 2′-biphenyldiol (=Bilevon) revealed a considerable scolicidal action.

Of miscellaneous drugs excepting salicylanilide- and bisphenol-derivatives, bunamidine hydrochloride revealed a high scolicidal effect, followed in order by

3-trifluoromethyl-4, 4′-dichlorocarbanilide and 3, 4, 4′-trichlorocarbanilide of halogenated carbanilide derivatives and dithiazanine iodide of cyanine dye. The scolicidal effect of nitroxynil, thiabendazole and parbendazole was generally low.

The survival rates of protoscoleces were tested in two media in which sodium carboxymethyl cellulose or propylene glycol were dissolved at the rates of 0.3 or 0.5 per cent, respectively. The survival time of protoscoleces in the medium having the former was slightly longer than that in the medium with the later as shown table 2. The comparative effects of propylene glycol and sodium carboxymethyl cellulose as additives in media were tested with the three bisphenol derivatives, 2, 2-sulfinylbis (4, 6-dichlorophenol) (=bithionol sulfoxide), 2, 2′-methylenebis (4-chloro-6-nitrophenol) and 2, 2′-methylenebis (3, 4, 6-trichlorophenol) and the two salicylanilide, 3, 5, 3′, 4′-tetrachlorosalicylanilide and 2′, 5-dichloro-4′-nitrosalicylanilide (=niclosamide). The scolicidal effect of these drugs suspended in the medium containing sodium carboxymethyl cellulose was evidently lower than that of the drugs dissolved or suspended in propylene glycol as shown in tables 3 and 4 and figure 3.

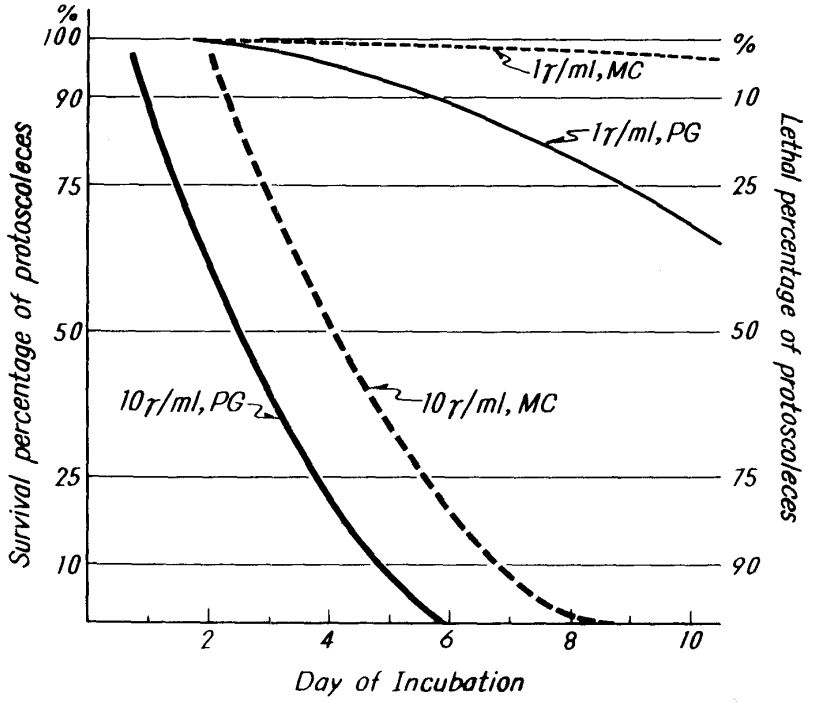
TABLE 4 *LT_{50} and LT_{90} of drugs for protoscoleces in two media containing propylene glycol and sodium carboxymethyl cellulose respectively*

DRUG	LT_{50} DOSE/ml			LT_{90} DOSE/ml		
	1γ	10γ	100γ	1γ	10γ	100γ
BISPHENOL DERIVATIVES	day	day	day	day	day	day
2, 2′-Sulfinylbis (4, 6-dichlorophenol)	12.97 (82.13)*1	2.50 (4.18)	++++*2 (++++)	17.92 (112.49)*1	4.74 (6.85)	++++*2 (++++)
2, 2′-Methylenebis (4-chloro-6-nitrophenol)	7.20 (10.16)	3.09 (4.64)	++++ (++++)	10.19 (17.42)	5.72 (−)	++++ (++++)
2, 2′-Methylenebis (3, 4, 6-trichlorophenol)	2.75 (3.77)	0.93 (2.50)	++++ (++++)	6.27 (6.87)	4.58 (5.08)	++++ (2.34)
SALICYLANILIDE DERIVATIVES						
3, 5, 3′, 4′-Tetrachlorosalicylanilide	++++ (3.40)	++++ (0.46)	++++ (++++)	++++ (−)	++++ (4.72)	++++ (++++)
2′, 5-Dichloro-4′-nitrosalicylanilide	++++ (++++)	++++ (++++)	++++ (++++)	++++ (++++)	++++ (++++)	++++ (++++)

*1 Numbers parenthesized show the lethal times in medium with sodium carboxymethyl cellulose.

*2 In cases where quadratic equation was unable to be computed, the lethal times while survival percentages become less than 50 or 10 per cent, are expressed by 5, 4, 3, 2 and 1 plus signs in order of time on the 2nd, 4th, 6th, 8th and 10th days of incubation, respectively. The things which the survival percentages are more than 50 or 10 per cent by the 10th day are indicated by dash.

FIGURE 3 *Scolicidal effect of 2, 2'-sulfinylbis (4, 6-dichlorophenol) in two media containing propylene glycol and sodium carboxymethyl cellucose respectively*



MC: Sodium carboxymethyl cellulose
PG: propylene glycol
Regression line: Quadratic equation

····· 1 γ/ml, MC: $y = -0.006x^2 - 0.085x + 99.699$
—— 1 γ/ml, PG: $y = -0.226x^2 - 1.115x + 102.387$
- - - 10 γ/ml, MC: $y = 1.909x^2 - 36.020x + 167.204$
—— 10 γ/ml, PG: $y = 2.290x^2 - 34.477x + 121.954$

Discussion

The most important problem in the assessment of in vitro screening test is to select an appropriate criterion that indicates the intensity of scolicidal activity of drugs. Schwabe et al. (1963), Lukashenko et al. (1970, 1971) and Kovalenko (1971) assessed the activity of the scolex by its respiratory rate. Meymerian et al. (1963) and Frayha et al. (1971) used the relative scolicidal value $\left(\frac{LD_{50}}{CT}\right)$. This was calculated by dividing its LD_{50} for mice by the product of the lowest effective concentration (C) and the minimum effective exposure time (T). This scolicidal value takes account of toxicity to the host.

As a preliminary to the present study, the lethal concentrations required for mortalities of 50 and 90 per cent of protoscoleces (LC_{50} and LD_{90} for protoscolex) on

some drugs were estimated by means of the logarithmic-probit graphic method[1,2] as shown in table 3. These indices, however, were not always ideal for indicating the scolicidal effect of many drugs in the in vitro screening test on the grounds that these values varied sometimes within wide limits over time.

The rates of survival protoscoleces observed in the three concentrations of drugs, 1, 10 and 100 γ/ml, were transformed to take account of the survivors in the controls. The regression curves for the survival percentage over time (time-mortality curves) and LT_{10}, LT_{25}, LT_{50}, LT_{75} and LT_{90} for protoscolex were estimated by an appropriate computer program. These provide data for planning in vivo experiments to compare the toxicity of the drug to the parasite independently with that to host. These indices are comparable with knockdown time and survival time (KD_{50} & D_{50})[19] which are represented by the length of exposure time for knock-down and mortality of 50 per cent of insects tested in the specific concentrations of insecticide.

With regard to the in vitro scolicidal effects of drugs tested, these are similar to the results obtained with protoscoleces of *E. multilocularis* by SAKAMOTO et al. (1968) and SAKAMOTO (1973). With both species, it was found that salicylanilide derivatives produced the highest efficacy, next in order included derivatives of diphenyl sulfide and diphenylmethane, bunamidine hydrochloride and dithiazanine iodide. Besides those derivatives, some halogenated carbanilide derivatives gave strong effects.

Some relationships between the chemical structure and the scolicidal action of drugs were observed. Namely, the intensity of scolicidal action of salicylanilide derivatives showed a tendency to increase with the addition of halogen atoms such as chlorine and bromine. 3, 5-dibromo-3′-trifluoromethylsalicylanilide gave strong scolicidal effects. Of salicylanilide derivatives having halogen atoms in their same positions, chlorinated derivatives showed the highest scolicidal effect, followed by the derivatives with bromines, and the derivatives with iodines gave the lowest action. 3, 5, 4′-tetrachlorosalicylanilide with a chlorine on its anilide base was weaker than 3, 5, 3′, 4′-tetrachlorosalicylanilide with two chlorines on the base and 3, 5-dichloro-4′-nitrosalicylanilide with a nitro group on the base. 2′, 5-dichloro-4′-nitrosalicylanilide (=niclosamide) showed the most effective action of all. From the above findings, it is considered that the halogen atoms and nitro group on the anilide base of salicylanilide derivatives influence the intensity of in vitro scolicidal action. SAITO et al. (1963) reported that the anthelmintic action of salicylanilide derivatives having halogen atoms in their salicyl base against *Fasciola hepatica* was stronger than that of the compounds with halogen atoms on their anilide base, but the number of halogen atoms in the compounds did not have as much influence on intensity of anthelmintic action. The present results are not in full

accord with their results concerning the number of halogen atoms in the compounds and intensity of the anthelmintic action.

The investigators PARKE, DAVIS and Company (1968) using chemical assay procedures and radioisotope tracer techniques traced the metabolism of 4′-chloro-3, 5-diiodosalicylanilide acetate ester (=Clioxanide) in sheep. They concluded that 4′-chloro-3, 5-diiodosalicylanilide with free phenol (absorbable, metabolite I) was formed by hydrolysis of the o-acetyl linkage in Clioxanide, and subsequently 4-chloro-2′-hydroxy-3, 5-diiodosalicylanilide with two hydroxyl group (metabolite II) by hydroxylation. The in vitro scolicidal effect of Clioxanide may increase in similar way.

There was an apparent relationship between the number of halogen atoms of bisphenol derivatives and the intensity of the scolicidal action of the compounds, except for bithionol which was extremely highly active. 2, 2′-methylenebis (4-methylphenol) was ineffective. Sulfonylbis (2, 3, 6-trichlorobenzene) and methylenebis (3, 4-dichlorobenzene) were ineffective. The scolicidal action of 4, 4′-thiobis-(2, 6-dichlorophenol) was much weaker than that of bithionol. MURAKOSHI et al. (1969) demonstrated that the inhibitory action of bithionol on fumarate reductase in carbohydrate metabolism was due to its hydroxyl group. From the observations in the present experiment, it is conceivable that the presence of halogen atoms and hydroxyl group and their relative positions in the bisphenol derivatives determines the scolicidal activity.

Bunamidine hydrochloride in the medium containing propylene glycol showed a higher scolicidal action than when suspended in sodium carboxymethyl cellulose (SAKAMOTO et al. 1973). Bithionol sulfoxide, 2, 2′-methylenebis (4-chloro-6-nitrophenol), hexachlorophen, 3, 5, 3′, 4′-tetrachlorosalicylanilide and niclosamide showed stronger action in propylene glycol than in sodium carboxymethyl cellulose. It is conjectured that the absorption of salicylanilide- and bisphenol-derivatives through the tegument of protoscolex is assisted by the presence of propylene glycol.

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