SOME ANTHELMINTIC PROPERTIES OF OCTYLCHLORORESORCINOL AGAINST ASCARIS LUMBRICOIDES IN MAN*

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Among a series of highly effective alkylchlororesorcinols against *Ascaris lumbricoides*, effects of which were discovered in our laboratory in 1948/49 (1,2,3) as a result of systematic studies with the co-operation of Dr. Tomita and Dr. Uyeo (4,5,6) of the Pharmaceutical Institute of Kyoto University in the synthesis of these compounds, a member that proved lowest in toxicity as well as in local irritant action was 4-*n*-octyl-6-chlororesorcinol. Ashikaga (1) of our laboratory first studied clinical effects of this compound in 1949 and obtained satisfactory results, which, in due time, were confirmed by Hattori and his co-workers (7). For about a year after this there came forth no significant report on clinical studies of this compound. But, last year, reports by Hattori *et al.* (8) and by Inouye *et al.* (9) came out, and according to these reports, the efficacy of the compound seems to have suffered rather a severe diminution. Not only that but also, we too have discovered, after having examined two kinds of tablets of this compound prepared by a pharmaceutical Co., that, to our surprise, there occurred a great deal of deterioration in its efficacy compared to earlier data. Whereupon, we again examined the efficacy of three samples of crystalline octylchlororesorcinol that were synthesized since its first trial and found every one of them to be unsatisfactory.

In order to know to what causes this change could be ascribed, we decided to launch our researches in the following two directions: Firstly, to find out if any drug-resistance against this compound had been developed on the part of *Ascaris* under the recent circumstances in which hexylresorcinol became so popular and widely used, and secondly, to re-investigate the problems concerned with the purity of the compound. On the first point, however, recent Mannami's investigations (10) of actual cases revealed no convincing evidence of manifestations of drug-resistance that would explain the above-mentioned change in the efficacy. In respect to the second point, however, the analysis performed by Tomita *et al.* of Kyoto University showed, oddly enough, that the one lower in efficacy to be the purer. Puzzled by these findings, we finally asked them for a new sample of this drug to be prepared under the same condition as when we received it for the first time. The newly prepared crystals, when tested, proved to be much more efficacious than the other low-efficacy crystals, though the former had lower

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melting point than the latter.

Confronted with these facts, it became necessary for the authors to clarify as to what difference or differences in the properties of these two samples, which are supposed to be alike, the distinct difference in the behavior of these samples could be attributed. In this paper, the authors tried to solve the problem by studying if there is any difference in the physical and chemical properties of all samples of octylchlororesorcinol that have been used in the clinical investigations and by comparing pharmacologic properties of the low-efficacy crystals with those of the newly prepared efficacious crystals.

METHODS

The crystals of octylchlororesorcinol in this report are numbered for convenience' sake in their order of age of synthesis.

Clinical experiment: The drug was given in one dose with a glass of water early in the morning on an empty stomach and thereafter no food was permitted for three to four hours. No laxative was used. Excepting experiment III in which one and a half as much doses were administered, the routine dosages were set as follows: For adults and children over 12 years of age 1.0 g; for children 8 to 12 years of age 0.8 g; for children up to 7 years of age one-tenth g of age in years. Crystals were taken as they are or wrapped in a wafer by children up to 10 years of age; in other cases in gelatine capsules. The effects were estimated by two methods; egg-counting by Stoll's technique (11) and worm-counting, except in one group in which only egg-counting method was used (cf. note for Table I). Side effects were studied by inquiring each patient except children in which case reports of their parents were relied upon.

Diazo-reaction: It was performed according to Trim's method (12) partly modified by Kuroda (13) in our laboratory. This method is very sensitive toward chloroalkylresorcinols for which other methods of estimation are unsatisfactory. The method is as follows: Dissolve 0.01 M of sulfanilic acid in 100 cc of 1/2 N HCl. To diazotize, to every 2 cc of this solution add one drop of 0.5 per cent NaNO_2 and mix well. Thereafter the mixture should be kept cool with ice. Then for each 1 cc add one drop of saturated urea and shake the solution vigorously to remove the excess of nitrite. To estimate octylchlororesorcinol, take 1 cc aliquots of its solution (suitable concentration is between 1:600 and 1:10,000) in test tubes and add 2 cc of the above prepared solution. After mixing the two thoroughly, add 3 cc of 8 per cent NaOH. Bright orange color will develop instantly. Allow it to stand for fifteen minutes in 20°C water bath if the room temperature is below 20°C. Then add enough distilled water to make up its volume to 10 cc, and examine the intensity of the color by Pulfrich's photometer. For filter we used S. 53. Diazotization of sulfanilic acid and the manipulations necessary afterwards should be done on the very day of each experiment. Other methods shall be described as

we come to them.

RESULTS

A. Clinical experiments with octylchlororesorcinols of various melting points

Results of ten clinical experiments, classified according to different samples of crystals (or tablets in some cases) used each time, undertaken in our laboratory concerning the anti-ascaris effect of this compound are summarized in Table I. One will notice here that all crystals that are low in efficacy show unanimously high melting points, i.e. over 53°C. On the other hand, those which are effective all show low points. An exception was No. 6 which has been synthesized recently. Although No. 6 possessed lower melting point than No. 1 and No. 2 which were prepared originally, the former was not so effective as the latter.

When the side effects of the drug such as unpleasant feeling in abdominal area, headache, dizziness, etc. which appeared more or less in each group were compared, no significant difference due to difference in crystals was observed. Also, it cannot be said that there were less complaints when the crystals were of low efficacy such as No. 3, No. 4 and No. 5. However, we can say with conviction that any of the octylchlororesorcinols causes far less side effects than hexylresorcinol. According to a report of Ashikaga, Kume and others (14) who experimented with cyclohexylchlororesorcinol, the purgative action of this series of drugs seems to aid in the expulsion of worms. Accordingly a record of bowel movement of every patient was kept and examined. The results showed that there were greater tendency toward purgation in the experimental group I, II, III, and X in which the effect of the drug was greater than in the other groups. This fact is worthy of notice.

B. Pharmacological comparison between effective and low-efficacy octylchlororesorcinol crystals

Choosing No. 6 as an example of effective crystals and No. 4 as one of low-efficacy crystals, a few comparative experiments were carried out on the toxicity, local irritant action, and ascaricidal property.

1. Toxicity

Tragacanth emulsions containing these samples of crystals were administered by stomach tube to mice which were made to fast from the evening before and the deaths that occurred during that day and the next six days were observed. The results are shown in Table II. The mice on which both samples were used were bought at the same time and bred under the same conditions for more than two weeks. Besides, the same dose of each sample was administered at the same time. From the results it appears that No. 6, though very slightly, is higher in toxicity than No. 4.

TABLE I. Results of ten clinical experiments on effectiveness against *Ascaris* of octylchlororesorcinols of various melting points

Experiment	Samples under investigation		Patients		Eggs based on one gram formed feces		Per cent egg-count reduction
	Crystals or tablets	Melting point (°C)	no. of cases	Age	Before treatment	2-3 weeks after treatment	
I	C. No. 1	49.5-51.5	12	10-15	85,900	550	99.3
II	C. No. 2	51-53	12	9-18	311,850	2,400	99.2
III**			12	8-17	189,500	400	99.8
IV	T. No. 1	(53.5-54.5)	14	17-23	112,800	71,950	36.2
V	T. No. 2	(53.5-55.5)	13	16-45	114,000	93,450	18.0
VI	C. No. 3	53-55.5	8	5-27	156,350	47,800	63.4
VII	C. No. 4	54.5-57	13	4-47	137,850	39,000	71.7
VIII	C. No. 5	55-57.5	11	4-6	175,500	50,600	71.5
IX	C. No. 5	"	10	18-23	77,600	71,400	7.9
X	C. No. 6	45.5-49.5	14	4-50	332,950	47,500	85.7

Note: * Worm counting: In the experiment I, II and III, all feces of patients passed during 10 days were examined by the experimenter himself staying together with them; in the experiment IV and V, the same method but during 5 days; in the VI, VII and VIII, data were based on the parents' surveys for 7 days; in the IX, not examined; and in the X experiment, the patients were ordered to bring all feces by themselves every day for a week.

** 50 per cent more than the routine dosage.

TABLE II. Comparison of oral toxicity to mice between No. 6 (moderately effective crystals) and No. 4 (low-efficacy ones)

Dose (mg/10g)	Crystals	Day and no. death								no. killed
		1	2	3	4	5	6	7	Total	
8	No. 6	0	0	0	1	1	0	0	2	2 / 6
	No. 4	0	1	0	0	0	0	0	1	1 / 6
10	No. 6	5	3	4	0	1	0	0	13	13 / 14
	No. 4	5	2	0	1	0	0	0	8	8 / 14
12	No. 6	4	1	0	0	0	0	0	5	5 / 6
	No. 4	2	2	0	0	1	0	0	5	5 / 6

2. Local irritant action

a. *Human tongue*: Upon each side opposite to each other of the tongues of five volunteers whose stomachs were empty was put almost simultaneously a drop of tragacanth emulsion of respective sample of crystals or a minute amount of respective crystals themselves, and each subject was asked how he felt. The emulsion concentration of 1:50 of either sample elicited no definite irritation. When

it was increased to 1:25 a sort of a irritating sensation was felt by subjects with No. 6 both in emulsion form and in free crystalline state. With No. 4 no such nsensatio was experienced.

(Continued from the left table)

Per cent patient who became negative in egg	Per cent patient worm in feces*	Average no. of expelled worms per patient	Date of experiment	Experimenter
83.3	91.7	1.9	Aug. 1948	Ashikaga
66.7	100.0	12.1	Sept. 1948	"
75.0	100.0	13.1	"	"
0	14.8	0.14	Apr. 1950	Mayeda
0	15.4	0.23	"	"
0	12.5	0.38	Jun. 1950	"
9.1	15.4	0.38	"	"
9.1	36.4	0.64	"	"
0			"	"
95.7	83.3	4.5	Jan.-Feb. 1951	Mannami

b. Conjunctiva of a rabbit: After having instilled 0.2 cc of an emulsion of respective sample, both in the same concentration, to the left and right conjunctival sacs respectively of a rabbit, it was kept there for one minute. Then any change in symptoms that occurred with passing of time was observed. Table III shows the results.

TABLE III. Comparison of local irritant action on the conjunctiva of rabbits between No. 6 (moderately effective crystals) and No. 4 (low-efficacy ones)

Concentration	Rabbit No.	Crystals	Time after instillation								
			15 ^m	30 ^m	1 ^h	2 ^h	5 ^h	24 ^h	48 ^h	72 ^h	96 ^h
1 : 1000	1	No. 6	-	-	-	-	-	-	-	-	-
		No. 4	-	-	-	-	-	-	-	-	-
1 : 500	2	No. 6	L ±	-	L ±	-	H+, S+	S+	-	-	-
		No. 4	-	-	-	-	H+, S+	S ±	-	-	-
	3	No. 6	L ±	-	-	H+	H+, S ±	H+, S+	-	-	-
		No. 4	L ±	-	-	H+	H+, S ±	H+	-	-	-
1 : 250	4	No. 6	L+, H+	L+, H+	L ±, H+	H+, S ±	H+, S+	H+, S ±	H+	H+	-
		No. 4	L+, H+	H+	L ±, H+	H+	H+, S+	H+, S ±	H+	H+	-
	5	No. 6	L+	L ±	H+	H+, S ±	H+, S ±	H+	H+	H ±	-
		No. 4	L+	L ±	H+	H+, S ±	H+, S ±	H+	H+	H ±	-

Note: L) lacrimation; H) hyperemia; S) secretion of Meibomian glands;
-) none; ±) slight; +) marked

From these data, it seems that No. 6 is more irritant than No. 4, but the difference is very little.

3. *Ascaricidal action in vitro*

Each of the two samples of octylchlororesorcinol, after having been mixed with same amount of acacia and ground together, was suspended in Bunge's solution to the concentration of 0.1 per cent. 50 cc of each drug suspension was poured out into a 200 cc beaker and these beakers were dipped in a constant temperature bath of $37.5 \pm 0.5^\circ\text{C}$. Then, a fresh human *Ascaris* that had been expelled within 29 hours by the effect of santonine and thoroughly washed by Bunge's solution—such worms were vigorous and lived just as long as those taken out of intestine by operation in Bunge's solution (15), was put in each beaker and the duration which the drug required to deprive of its motility was measured. In this experiment, we employed two kinds of stimuli in order to examine the response of the worms. One is what we call a mechanical stimulus, which consists merely in stirring the worm body together with liquid by a wooden chopstick, and/or stroking the same along its full length with it. Then, when they became inactive, i.e. showing no response to these stimuli, they were made to come in contact at a definite interval (10 minutes in the present experiment) with hot water of temperature 60°C for a brief moment (practically less than two seconds). This latter kind of stimulus is what we call a heat stimulus. It was applied until the worm did not show any response to it when it was considered dead. In this experiment, we employed freshly prepared drug suspensions, because alkylresorcinols are apt to change in Bunge's solution due to its alkaline nature. The experimental results are shown in Table IV. It can be seen that compared to No. 4, No. 6 kills the worm more swiftly.

TABLE IV. Comparison of ascaricidal effect *in vitro* between No. 6 (moderately effective crystals) and No. 4 (low-efficacy ones)

Crystals	Human <i>Ascaris</i>			Time of exposure to 1:1000 concentration of drug suspension required to stop reaction toward stimuli	
	no.	♂ : ♀	length (cm)	Mechanical stimulus (min.)	Heat stimulus (60°C) (min.)
No. 6	15	11 : 4	14-23	64 ± 1.77	95.8 ± 4.10
No. 4	10	5 : 5	14-23	106 ± 11.27	134.5 ± 8.36

When all above experiments are considered together, one will find that those effective crystals, though slightly more toxic and more irritant in their nature, possess a greater annihilating power for *Ascaris* than the low-efficacy crystals.

C. Differences in physico-chemical properties observed among various samples of octylchlororesorcinol

1. *Melting point*

As stated before, the crystals that are effective all possessed melting points below 53°C, while those possessing melting points higher than this all proved to be low in efficacy or practically ineffective. The effectiveness of the crystals seems to deteriorate in the course of purification. But, the effectiveness may not be dependent only upon the lowering of melting point, for the crystals No. 6, though they have a lower melting point than either No. 1 or No. 2 never showed effectiveness greater than the latter.

2. *Color*

Excepting snow white crystals No. 5, all others more or less had chocolate color, the degree of which was in the following order: No. 1 > No. 2 > No. 6 > No. 3 > No. 4 > No. 5. This color is presumed to be the result of gradual changes in color with passing time generally observed in phenols. The results showed that the efficacy of crystals is in direct proportion to the intensity of color, i.e. the more effective the more distinctly colored, but it has no relation to the age of crystals. This phenomenon, alike that of melting point, suggests that more effective the crystals are, the less pure they are.

3. *Crystalline form*

When examined microscopically, each of the six samples of crystals belongs to one of three categories of crystalline forms represented by No. 1 (all fine crystals), No. 6 (for the most part fine crystals and in part large ones), and No. 5 (all large crystals) (cf. Fig. 1) or to their intermediate forms. For example, the crystalline

FIG. 1. Microscopic pictures of two kinds of crystals of octylchlororesorcinol, a: Large crystals (No. 5); b: Fine crystals (No. 1). $\times 500$

FIG. 2 X-ray powder photographs of two kinds of octylchlororesorcinol: No. 1 (a) and No. 5 (b), and octylresorcinol (m. p. 74-75°C) (c)

form of No. 2 lies between No. 1 and No. 6; that of No. 3 between No. 5 and No. 6; and that of No. 4 almost same as No. 5. If we observe these facts in connection with their effects, one will notice that the more effective the crystals are, the greater the number of the fine crystals contained in them. However, it would seem more proper to think that the form of crystals might be influenced by some fundamental factors upon which their effect depends, rather than to ascribe this remarkable difference in effect to the crystalline size.

Crystals No. 1 and No. 5 were subjected to X-ray analysis by the aid of the Pharmaceutical Institute of Kyoto University. According to the results obtained, these two samples are identical from the standpoint of lattice constants and, consequently, crystallographically speaking, they belong to the same class of crystals. In octylresorcinol (lacking chlorine) conspicuous reflection is seen at $d=3.59 \text{ \AA}$, though in the photographs of No. 1 and No. 5 there can be found no trace of it (Fig. 2). Studies with polarizing microscope revealed that all crystals composing No. 1 as well as No. 5 belong to a monoclinic system, and there can be detected

no orthorhombic crystals, characteristic of octylresorcinol, in them. The only possible difference between the two samples of octylchlororesorcinols may be that there is some amorphous substance in No. 1 which is lower in melting point.

4. Solubility and turbidity

As regards the approximate solubility of three samples of crystals, i.e. No. 4, No. 5 and No. 6, in water at 25°C, that of former two was 1: 140,000, while that of No. 6 1: 70,000. While the former two can be dissolved to form almost a transparent solution, the solution of latter has a tendency to become turbid. In 25 cc volumetric flasks were put in 250 mg of No. 4, No. 5 and No. 6 crystals respectively, and then they were filled up to the volume by adding hot (70°C) distilled water, thus making the concentration of the solutions 1 per cent. After they were shaken thoroughly they were allowed to stand for 48 hours at room temperature (4-10°C). After filtering to separate crystalline residues, the filtrates were compared for transparency. The filtrates of No. 4 and No. 5 were almost transparent but that of No. 6 showed much turbidity. By means of Pulfrich's photometer, transmission coefficient was determined using S. 53 filter. The values obtained were 98 for No. 5, 96 for No. 4 and 67 for No. 6.

5. Interfacial tension

The ability of foaming in a similar concentration seemed to be slightly stronger in solutions of No. 1, No. 2, and No. 6 compared to those of others. So, in 1: 200,000 solution of each sample of crystals measurement of surface tension was made by du Nouy's method. Results are shown in Table V. As a matter of fact, every sample showed considerably strong surface activity, but it must be noticed that those effective ones, No. 1, No. 2 and No. 6 occupy much higher places in this regard than the others. Still more interesting was the fact that, in proportion to the rise in melting point, the surface activity decreased regularly. To investigate this phenomenon further, using 1 per cent filtrate prepared as stated in the preceding chapter, a comparison was made between No. 4 and No. 6. Here the differences came out more distinctly (Table V).

TABLE V. Surface tension of six samples of crystals at 10°C

Crystals	Concentration	Surface tension (dynes/cm)	Depression of surface tension (dynes/cm)
No. 1	1 : 200,000	47.64	26.58
No. 2	"	46.28	27.94
No. 3	"	52.74	21.48
No. 4	"	54.18	20.04
No. 5	"	54.66	19.56
No. 6	"	47.48	26.74
No. 4	1 : 100 (filtrate)	50.47	23.75
No. 6	"	31.17	43.05

TABLE VI. Interfacial tension between liquid paraffin and the filtrates of 0.1 per cent solution at 25°C

Crystals	Interfacial tension (no. of drops)
No. 1	10.6
No. 3	8.3
No. 4	7.2
No. 5	7.4
No. 6	10.3

Note: The amount of No. 2 was not sufficient for the experiment

each of the samples to the results observed on the surface (air/water interfacial) activity.

6. Diazo-reaction

The color was made to develop in four samples of crystals, namely No. 1, No. 6, No. 4 and No. 5, the former two being crystals of high efficacy and the latter two of low efficacy, by diazo-reaction and the relation between the intensity of

FIG. 3. Calibration-curves of various preparations of octylchlororesorcinol by the diazo-reaction

Since the surface of *Ascaris* is known to be hydrophobic, in order to simulate the biological condition more closely, the interfacial tension between liquid paraffin and the aqueous media consisting of the filtrates of 0.1 per cent solutions were measured by means of Hillyer's stalagometric method (16). The measurements at 25°C are given in Table VI in terms of number of drops, which shows parallelism in the relation between

color and the concentration was studied by plotting extinction-concentration curves. For this purpose, we used specimens dissolved in 50 per cent alcohol. In this experiment, the color of all specimens was developed under the same condition of temperature which was 20°C and time which was 15 minutes. Also sample No. 1 was paired off with No. 5, and No. 6 with No. 4, and the color of each pair was made to develop simultaneously; and similar concentration of each pair was examined in turns one immediately following the other. Fig. 3 indicates the results of repeated measurement, showing extinction-concentration curve for each sample. One can see in this figure that the extinction-concentration curves of No. 1 and No. 6, which are high efficacy crystals, are

much higher than those of No. 4 and No. 5, which are low efficacy crystals. Yet, the color was the same orange in all cases.

Next, we took 1 cc of each of the 1 per cent solutions (filtrates) of three samples of crystals, viz. No. 4, No. 5 and No. 6, prepared under paragraph C-4 above, and the color was made to develop in each of them. The extinction-coefficients of these solutions are shown in Fig. 3 in which they are marked ○. It can be seen that while no color developed in No. 5, No. 4 was slightly tinted and No. 6 developed a distinct color, which was reddish orange. Next, after having centrifuged these solutions for half an hour at 3,000 revolutions per minute, the supernatant liquid was taken out gently and was examined; the results are those marked □. The concentrations of these solutions corresponding to their respective extinction-coefficients were estimated and the results are shown in Table VII. If we consider No. 5 as pure octylchlororesorcinol and assume that crystals of the same purity are contained in solutions of No. 4 and No. 6, then the concentrations of these solutions become so high as 18 mg per cent in No. 4 and 75 mg per cent in No. 6. If we recall the fact that the crystals of No. 4 and No. 6 could not be completely dissolved unless they were diluted above 1:70,000 (25°C), the high values we have obtained become hard to understand. Therefore, we must assume that the substance that developed color by the diazo-reagent in the filtrate and the supernatant liquid of 1 per cent solution is something other than pure octylchlororesorcinol. If our assumption is correct, then we can better understand the fact that while No. 5 whose melting point is highest of all, develops no color, while the color of No. 4 and No. 6, whose melting points descend in this order, becomes deeper in the same order.

TABLE VII. Apparent estimates of octylchlororesorcinol in the supernatants of 1 per cent solution of three kinds of crystals, No. 5, No. 4 and No. 6 by the diazo-reaction

Crystals	Extinction-coefficient of the supernatants	mg % calculated by extinction-concentration curves	
		as respective crystals	as pure octylchlorores. (No.5)
No. 5	0	not detectable	not detectable
No. 4	0.16	17	18
No. 6	0.72	45	75

If we mix a certain amount of octylresorcinol with No. 5, the extinction-coefficient rises, but relative amount of the former in the mixture had to be raised to as high as 15 to 20 per cent in order to give the coefficients corresponding to No. 1 and No. 6. According to the results of X-ray analysis, such can not be believed. Moreover Mannami (17) has proved in his recent clinical experiment that the effect of octylchlororesorcinol of high melting point cannot be markedly strengthened by

mixing 10 to 30 per cent of octylresorcinol. Mixing of caprylic or capronic acid to this does not increase the intensity of coloration.

DISCUSSION

As for the features of crystals of octylchlororesorcinol which are especially effective clinically, first we must note the fact that all of them have comparatively low melting points, namely below 53°C. This fact coincides well with the fact that, generally speaking, color of the effective crystals are deeper than that of the ineffective ones. We are obliged to accept the seemingly odd phenomenon as truth that the greater the efficacy the less pure the compound is, a fact out of harmony with our every day experience. There is a tendency of finer crystals to be contained in effective crystals, but according to studies with polarizing microscope as well as X-ray analyses, the size of crystals has no relation to the crystal system. Therefore, we believe that the effective substances in all samples of crystals are the same, namely octylchlororesorcinol, and the more efficacious crystals of low melting points contain impurities which enhance the action of the main crystals. The impurities can be removed by repeated recrystallization in petroleum ether.

The fact that all the effective crystals are superior to ineffective ones in their power to lower the interfacial tension of a solution is worthy of notice. One can well imagine that there is a close relationship between this interfacial activity of a crystal and its efficacy. A crystal which has a strong surface activity apparently dissolves more easily in water than others. However, such solution is slightly opaque rather than transparent. Probably due to the above property, the effective crystals are more easily dispersed, causing an emulsion than others. The property seems to be of great advantage in attacking the worms; especially, this interfacial activity is believed to be of a help in making the surface of the worm cuticle to adsorb the drug. In fact, it was demonstrated in our experiment, that crystals of this compound with a stronger interfacial activity could kill the worm *in vitro* more rapidly than those with a weaker activity. We are much interested in the works recently reported by Alexander and Trim (18) who measured quantitatively the effect of surface active substances upon the penetration of hexylresorcinol into the pig round worm with parallel measurements of the interfacial activity of these solutions against an inert mineral oil. Using a fixed hexylresorcinol concentration the effect of different dilutions of soap solutions upon it was observed. A dilute soap solution accelerated the penetration of this drug, but when present in high concentration the penetration was completely inhibited. Measurement of interfacial tensions indicate that the soap and hexylresorcinol molecules may associate at the interface, forming a labile 'complex' of increased surface activity. The maximum interfacial activity (i.e. minimum interfacial tension), was found in all cases to occur at the soap concentration which produced maximum hexylresorcinol penetration into *Ascaris*. This particular concentration of soap solution corresponded to

the onset of micellar aggregation of the soap molecules; thereafter the drug distributes itself between the micelles and any other interface present. At high soap concentrations the hexylresorcinol is mainly held by the soap micelles so that the biological activity is diminished, ultimately reaching zero since the soaps alone penetrate so extremely slowly. This explanation suggests that the biological activity of hexylresorcinol and several other homologues is determined by the interfacial activity of the mixture.

The effective crystals induced more purgation in the patients than the ineffective ones. This action, too, would serve, to some extent, to expel the worms. Probably the increase in surface activity might have helped to augment the local irritant action of crystals, though not to a marked degree.

This increment of interfacial activity in the effective crystals of octylchlororesorcinol can no doubt be ascribed to minute amount of impurities mixed in with the crystals, the nature of which, to our regret, remains still uncertain at our recent state of research. The fact that when one couples octylchlororesorcinol with diazotized sulfanilic compound, the effective crystals develop a far more distinct color than ineffective ones in the same concentration, makes one suspect the existence of octylresorcinol which produces a stronger color than octylchlororesorcinol, mixed in with the effective crystals. Yet, under X-ray analysis and the other examinations, this surmise is unlikely to be the case; instead it seems that this impurity is an amorphous substance. Diazo-reaction would not be influenced by the presence of caprylic acid. This unknown substance is being investigated further. On the other hand, the question whether any other surface active agent can heighten the anthelmintic activity *in vivo* of this class of compounds cannot but stir a deep interest in us.

The question, however, naturally arises as to whether the increase in the effectiveness is due only to surface activity, for melting point is thought also to be an important factor in the anthelmintic activity [Harwood (19), Lamson and co-workers (20)]. It is probable that the effective crystals having lower melting points liquefy more easily and attain a beneficial state for attacking the worms in the host compared to the ineffective ones. But, this also may not be the only cause, because there cannot be seen strict parallelism between the effectiveness and the degree of lowering of melting point.

SUMMARY

1. Having realized that there occurs a great deal of difference in the effectiveness of crystals of octylchlororesorcinol as an anthelmintic according to how they were prepared, some experiments have been undertaken, in order to find out the cause, to study the biological as well as physico-chemical properties of the crystals that have been used in the clinical experiments.

2. All the crystals that were effective clinically showed low melting points,

i.e. below 53°C, while those above this degree were low in effectiveness or practically ineffective. Since crystallographically these different samples of crystals were found to be identical, it seems that the effective crystals contain as impurities some amorphous substances.

3. The effective crystals proved much stronger in interfacial activity than the others; undoubtedly this increment of the activity can be ascribed to the impurity.

4. Although the nature of the impurity remains still uncertain, it is known that it is easily removed by petroleum ether, relatively soluble in water, and develops color by the diazo-reaction probably more distinctly than octylchlororesorcinol itself, but differs from octylresorcinol or fatty acids.

5. The effective crystals annihilate *Ascaris* more swiftly than the others, and though to a slight degree are more toxic and more irritant.

6. On the basis of these findings, discussions were made as to the cause of differences in effectiveness of the crystals, with especial emphasis on the significance of the interfacial activity and the melting point.

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REFERENCES

- 1) YAMASAKI, H. & ASHIKAGA, M.: *Folia pharmacol. japon.* **44**, 30§ (1949).
- 2) ASHIKAGA, M.: *Ibid.* **44**, 32§ (1949).
- 3) YAMASAKI, H.: *Reports of Joint Research on Anthelmintics* (Western Group of the Developmental Scientific Research) **1**, 17 (1948); **2**, 33 and **3**, 19 (1949).
- 4) TOMITA, M., UYEO, S., KOBAYASHI, S., KOIZUMI, J. & TAMURA, K.: *J. Pharmaceut. Soc. Japan* **69**, 149 (1949).
- 5) TOMITA, M., UYEO, S., KOIZUMI, J., KOBAYASHI, S. & TANGE, G.: *Ibid.* **69**, 151 (1949).
- 6) TOMITA, M., UYEO, S., MORIMURA, H., KOBAYASHI, S., KOIZUMI, J., MAEDA, R. & FUKAGAWA, K.: *Ibid.* **71**, 73 (1951).
- 7) HATTORI, S., OKAZAKI, H., TAKAYANAGI, H. & KAKEMI, K.: *Reports of Joint Research on Anthelmintics* (Western Group of the Developmental Scientific Research) **2**, 54 (1950).
- 8) HATTORI, S., SUGIMOTO, S., EMI, I., OKAZAKI, H. & TAKAYANAGI, H.: *Ibid.* **5**, 97 (1950).
- 9) INOUE, K., MIYOSHI, A., ONISHI, K., HARADA, T. & OKUDA, H.: *Ibid.* **6**, 93 (1950).
- 10) MANNAMI, C.: *Folia pharmacol. japon.* **47**, 35§ (1951).
- 11) STOLL, N. R. & HAUSHEER, W. C.: *Amer. J. Hyg.* **6**, March Supp. 80 (1923).
- 12) TRIM, A. R.: *Parasitology* **35**, 239 (1944).
- 13) KURODA, A.: *Medicine and Biology* **23** (1951) (in the press).
- 14) ASHIKAGA, M., KUME, M., YANO, Y., KUCHII, I., MIYAKE, K. & YOSHIHARA, S.: *Saishin-*

Igaku **5**, 80 (1950).

- 15) ASHIKAGA, M.: *Folia pharmacol. japon.* **44**, 27§ (1948).
- 16) HULLYER, H. W.: *J. Amer. Chem. Soc.* **25**, 1256 (1903).
- 17) MANNAMI, C.: To be published.
- 18) ALEXANDER, A. E. & TRIM, A. R.: *Proc. Roy. Soc., London*, s. B. **133**, 220 (1946).
- 19) HARWOOD, P. D.: *Proc. Soc. Exper. Biol. & Med.* **32**, 131 (1934).
- 20) LAMSON, P. D., BROWN, H. W., STOUGHTON, R. W., HARWOOD, P. D., BALTZLY, R. & BASS, A. D.: *J. Pharmacol. & Exper. Therap.* **53**, 239 (1935).