and its subsequent enlargement for further protection. This collar would be a key event in the evolution of the future mantle and shell of the brachiopods. Water current through the collar would be improved by the formation of two slits which divide the collar into two "mantle lobes" (Fig. 5D). Collapse of these soft lobes would be prevented by the formation of a skeletal support—that is, the evolution of two external valves of organic and later mineralized material (Fig. 5E). The final stage in our model (Fig. 5F) is an animal with a two-valved shell protecting a tentacular cavity, and with a soft body containing a coelomic cavity and two dissepiments supporting two pairs of metanephridia and gonads and suspending the intestinal canal. The dissepiments are an important part of the mechanism by which lateral body swelling is prevented during hydraulic shell opening. The posterior part of the wormlike ancestor is retained as the pedicle; its metamerism is lost but the muscular wall around the coelomic tube and the cuticular coat are retained. Existing transverse muscles could insert directly on the shell, and some could function as adductor muscles. The shell-opening mechanism is achieved by an indirectly operating system formed by a complex of laterally placed longitudinal muscles, fluid-filled coelomic cavities, and transverse bands, all parts of the existing hydraulic locomotor system of the metameric wormlike ancestors. The morphology as abstracted in the final stage of our series is that of the *Lingula*-like Inarticulata described at the onset (Figs. 1A and 2).

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Cholestyramine: Use as a New Therapeutic Approach for Chlordecone (Kepone) Poisoning

Abstract. *In rats, as reported in humans, chlordecone (Kepone) is excreted predominantly in the feces. Cholestyramine, an anion exchange resin, binds chlordecone in rat intestine, increases its excretion into the feces, and decreases its content in the tissues. The resin appears to offer a practical method for treating chronic poisoning with this and possibly with other lipophilic toxins.*

Workers in a factory that manufactured chlordecone (Kepone) (*1*) were exposed to large quantities of this organochlorine pesticide for many months. The workers had high concentrations of chlordecone in their blood and fat associated with clinical evidence of toxicity to the nervous system, liver, and testes (*2*). No treatment is available for patients poisoned with organochlorine pesticides, such as chlordecone, except for measures aimed at relief of symptoms. One approach to therapy would be to accelerate the elimination of chlordecone from the body. This procedure is based on the assumption that the continued presence of the chemical in the tissues at high concentrations is necessary for overt toxicity and may also carry the potential for development of malignancy. The latter concern has been raised by the demonstration that chlordecone is carcinogenic in rats and mice (*3*).

In man chlordecone passes through the liver into bile, but only a small fraction of the biliary chlordecone entering the intestine appears in the stool (*2*). These observations suggest that elimination of chlordecone from the body is curtailed by reabsorption of the chemical from the contents of the intestine, creating an "enterohepatic recirculation" of chlordecone. If this hypothesis is correct, then the rate of elimination of chlordecone would be augmented by oral administration of a nonabsorbable agent that would bind chlordecone in the intestinal lumen. Cholestyramine, an anion exchange resin, binds chlordecone in vitro (Fig. 1). In short-term clinical tests of this resin, we have found that treatment of six patients with cholestyramine for 3 days produced an average sixfold increase in excretion of chlordecone in the stool (*2*). These results in patients are encouraging and suggest that a clinical trial of cholestyramine therapy is warranted. However, it seemed important to test first in animals the effect of the resin not only on fecal excretion but also on tissue content of chlordecone. Furthermore, the concentration of organochlorine pesticide may be underestimated in human samples since metabolites of chlordecone, if present, may escape detection by gas-liquid chromatography (GLC). Animals given ^{14}C-labeled chlordecone provide a ready means for studying the excretion of total organochlorine material and the stimulation of this process by cholestyramine.

Male Sprague-Dawley rats weighing 175 to 200 g were selected for study because the toxic manifestations of chlordecone in these animals (*4*) resembles those observed in man. The animals were housed individually in metabolic cages and allowed free access to food and water. After being fasted overnight, rats were given chlordecone (40 mg/kg) in solution by gastric tube. The solution of chlordecone was prepared by dissolving one part of [^{14}C]chlordecone (Pathfinder Labs, St. Louis, Mo.) with stated specific activity 19.68 mCi/mmole and 49 parts of unlabeled chlordecone (Allied Chemical) in warmed corn oil. The purity of both chlordecone products was greater than 97 percent, as judged by GLC and thin-layer chromatography. The total output of feces from the labeled rats was collected at 24-hour intervals. The stool was weighed and homogenized (20 percent, weight to volume) in distilled water. Portions (0.2 g) of the stool homogenates were vaporized (Packard 306 Tri-Carb Oxidizer) with the use of Carbosorb. Radioactivity was measured by liquid scintillation spectrometry with [^{14}C]toluene as an internal standard for counting efficiency. (Permaflow was used as counting fluid.) Urine, collected

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separately from the stool, showed negligible radioactivity throughout this study.

Less than 10 percent of the oral dose of chlordecone appeared in the feces during the first 24 hours (Fig. 2). During the next 7 days, the daily excretion of chlordecone in the stool in each animal was constant, being approximately 5 percent of the amount of chlordecone remaining to be excreted. This observation, as well as sequential measurements of the concentration of chlordecone in tissues after oral administration of [^{14}C]chlordecone (*4*), indicated that the distribution of the pesticide rapidly achieved equilibrium. Seven days after receiving chlordecone, the rats were treated orally with either Questran (44 percent anhydrous cholestyramine) or a placebo in which cholestyramine was replaced by 8 percent silica gel and 36 percent cellulose (Mead-Johnson). Collection of feces was continued. At the start of treatment, and at 7 and 14 days thereafter, the concentration of [^{14}C]chlordecone in the tissues was measured. With the rats maintained at 37°C and under anesthesia (urethane, 1.2 g/kg), the abdomen was exposed, and timed collections of bile were obtained from the bile duct, which was cannulated with P.E. 10 tubing. Next, the rats were killed by exsanguination, samples (20 mg) of various organs were removed, and the entire animal was digested in 250 ml of 10*N* NaOH. For determination of radioactivity, the samples of tissue and samples of the whole animal digest were oxidized and radioactivity was determined as described above for stool samples.

Table 1. Experimental conditions were as described for Fig. 1. After being treated for 2 weeks with either cholestyramine or placebo, rats were killed and biopsies of the indicated organs were removed, weighed (wet), and analyzed for [^{14}C]chlordecone. Data are given as the mean ± S.E.M. for duplicate determinations in six rats. With the exception of fat, differences in tissue [^{14}C]chlordecone between rats treated with cholestyramine or placebo are significant at $P < .05$. Although the percentage decreases tended to be highest in gut and brain, the differences in the decreases among all tissues did not differ significantly ($P > .05$).

Tissue	[^{14}C]Chlordecone concentration (dpm/g)		Decrease (%)
	Placebo	Cholestyramine	
Liver	188,684 ± 20,000	114,537 ± 12,000	39
Adrenal	64,375 ± 9,000	44,296 ± 3,100	31
Fat	32,719 ± 6,600	22,805 ± 2,600	30
Lung	45,062 ± 5,700	28,121 ± 4,800	37
Stomach	29,999 ± 3,000	14,286 ± 950	52
Small intestine	28,450 ± 3,000	13,582 ± 3,200	52
Colon	15,244 ± 1,500	9,226 ± 970	40
Brain	25,495 ± 2,200	12,427 ± 1,090	51
Bile	8,006 ± 570	4,891 ± 210	39
Blood	3,794 ± 300	1,991 ± 200	48

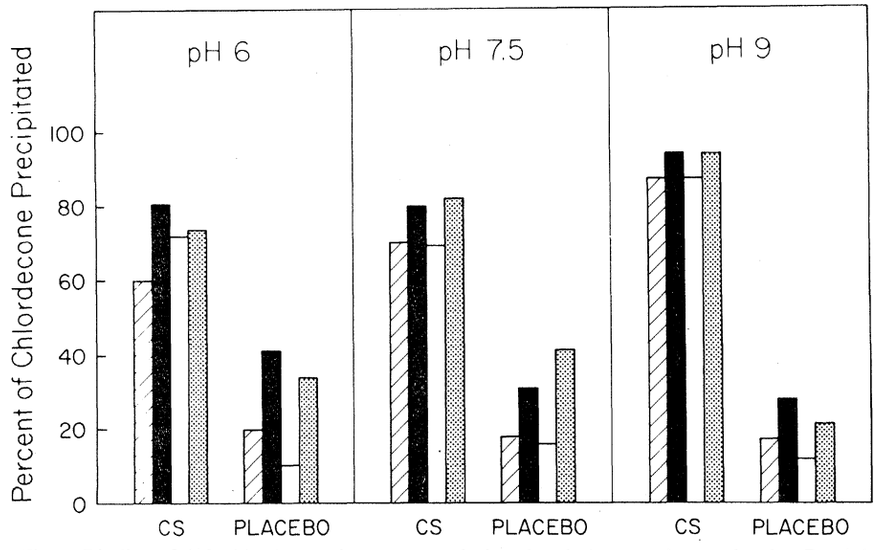

Fig. 1. Binding of [^{14}C]chlordecone in aqueous solutions by cholestyramine or placebo. Precipitation of [^{14}C]chlordecone from aqueous solutions at different *p*H values was studied using a modification of the method of Gallo (*10*). The incubation mixture consisted of 0.2 ml of 0.02*N* NaOH containing either 1 or 10 μg of [^{14}C]chlordecone, 1.8 ml of Trizma buffer (*p*H 9, 7.5, or 6), and 5 or 50 mg of either cholestyramine as Questran® or of placebo. The mixture was agitated briefly and shaken in a water bath at 37°C for 1 hour. After a second brief agitation, the solid material was sedimented at 1500*g* for 15 minutes, and the radioactivity in 0.50 ml of the supernatant was counted. Binding of chlordecone to the resins was calculated by comparing the amount of radioactivity remaining in the supernatant in incubations with cholestyramine or placebo to that in a control incubation containing only buffer and [^{14}C]chlordecone. The ratio of concentration of [^{14}C]chlordecone (micrograms per milliliter) and cholestyramine (*CS*) or placebo (milligrams per milliliter) in the test tube was as follows: hatched bars, 1:5; solid bars, 1:50; open bars, 10:5; dotted bars, 10:50. Each point was determined in duplicate. Washing the sedimented cholestyramine-chlordecone complex with an additional 1 ml of fresh buffer released only 2 percent of the total precipitated radioactivity into the supernatant. By contrast, the chlordecone precipitated with placebo was removed almost entirely by two washings with buffer.

For measurement of chlordecone chemically, some samples of tissue or excreta were acidified and extracted with a mixture of hexane and acetone. These extracts were analyzed for total radioactivity and for organochlorine substances by GLC (*5*). Chlordecone was the only compound identified by GLC in all samples from eight rats. However, indirect evidence for the presence of chlordecone metabolites was obtained by calculating the "apparent specific activity" of the extracted chlordecone. This value, expressed as disintegrations per minute per nanogram of chlordecone [mean ± standard deviation (S.D.)] [in blood (2.19 ± 0.3), fat (2.2 ± 0.3), and liver (2.17 ± 0.16)] was similar to that of the administered chlordecone (2.20), extracted from corn oil by the same procedure. By contrast, the apparent specific activities of chlordecone extracted from bile (2.56 ± 0.18) and stool (3.10 ± 0.18) were significantly higher. Recovery of radioactivity from the tissues and bile was 80 to 90 percent, whereas that from the stool was 64 percent. The increase in apparent specific activity of chlordecone extracted from bile and stool is best explained by postulating the presence of metabolites of chlordecone which escape detection under conditions optimized for measurement of chlordecone by GLC. Isolation of these metabolites is needed in order to validate this hypothesis.

In all animals, 96 ± 7 (S.D.) percent of the administered radioactivity was accounted for in body stores or in excreta at each point of killing. Cholestyramine produced an increase in the fecal excretion of chlordecone which was detectable within 24 hours (Fig. 1). The total excretion of pesticide in the stool during the 2 weeks of treatment with cholestyramine was twice that of control animals receiving placebo. Coinciding with stimulated excretion of pesticide, choles-

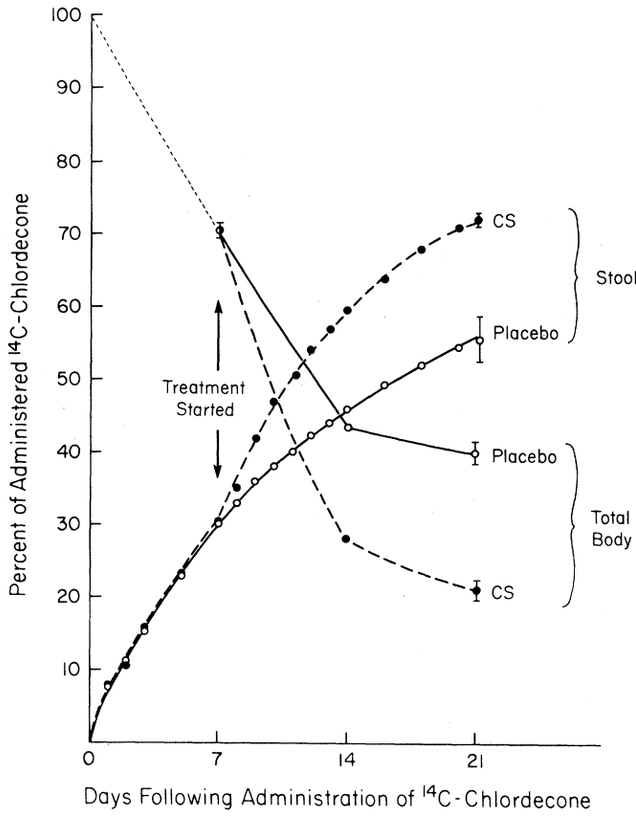

Fig. 2. Stimulatory effect of cholestyramine on excretion of [^{14}C]chlordecone in rats. Rats (20) were given a single dose of [^{14}C]chlordecone (40 mg/kg). The animals were divided into two groups. On day 7 of the study, the diet was changed from standard laboratory chow in powdered form to the same meal supplemented with 4 percent of cholestyramine (group 1) or placebo (group 2). The animals in both groups consumed equal amounts of food and gained weight similarly. Total radioactivity was measured in four rats on day 7, in a pair of rats for each treatment on day 14, and in six rats for each treatment on day 21. Cumulative excretion of radioactivity in the stool was calculated daily and is presented as the average for all rats remaining in the study. Variation in the data is given as the standard error of the mean (brackets). As calculated from the excretion rate of chlordecone in the stools, other losses having been found negligible, linear regression of the natural logarithm of the amount of chlordecone remaining to be excreted (from day 7 to day 21) yields rate constants for excretion of chlordecone of 0.065 per day and 0.033 per day for rats treated with cholestyramine or placebo, respectively.

tyramine produced an equal decrease in the total radioactivity in the animal. A similar decrease in radioactivity was observed in individual tissues of cholestyramine-treated rats (Table 1). The latter finding suggests that, in cholestyramine-treated rats, the increment in excreted chlordecone did not arise from a small, readily mobilizable pool but rather was derived from each tissue examined in proportion to the concentration of chlordecone in the tissue.

Total biliary excretion of [^{14}C]chlordecone in rats was not in excess of that appearing in the stool, in contrast to our findings in patients (*2*). Measurements of bile production and biliary [^{14}C]chlordecone excretion were made in rats after 14 days of treatment. Maximal bile flow was the same in rats treated with placebo [2.85 ± 1.3 ml/kg per hour (S.D.) $N = 5$], or cholestyramine [2.4 ± 0.6 ml/kg per hour (S.D.) $N = 5$]. Biliary excretion of [^{14}C]chlordecone by placebo-treated animals represented 103 ± 40 percent (S.D.) of the [^{14}C]-chlordecone excreted in the stool in the preceding 24 hours. For cholestyramine-treated rats, biliary excretion of chlordecone accounted for only 47 ± 10 (S.D.) percent of that excreted in the feces. In these calculations, the assumption was made that the maximal excretion of radioactivity in bile in one of three consecutive 30-minute collections was directly proportional to that excreted for 24 hours in the intact animal. Therefore, on the assumption that substantial amounts of biliary chlordecone are absorbed in the intestine, the data suggest that chlordecone enters the gastrointestinal tract from a second source, in addition to bile. Moreover, entry of chlordecone into the intestine by this postulated additional pathway is augmented by cholestyramine. Among many possibilities, this pathway could be desquamation of chlordecone-laden cells lining the intestine or transport of chlordecone into the lumen directly across the intestinal mucosa. The existence of a nonbiliary mechanism for excretion of other lipophilic hydrocarbons into the intestine may also be inferred from studies of the pharmacodynamics of mirex (*6*), dieldrin (*7*), and polychlorinated biphenyls (*8*).

On the basis of the present data we believe that cholestyramine represents promising therapy for patients with high levels of chlordecone and possibly for asymptomatic individuals with low concentrations of this pesticide. The resin has been used safely for many years to treat conditions that are ameliorated by binding bile salts in the intestine and thus interrupting their reentry into the hepatic circulation. However, the use of cholestyramine therapy need not be confined to substances excreted in bile. The resin has been reported to stimulate nonbiliary excretion into the intestine of such hydrophobic compounds as unconjugated bilirubin (*9*) and, in the present studies, chlordecone. Therefore, cholestyramine may offer a means for detoxification of patients exposed to various lipophilic substances.

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