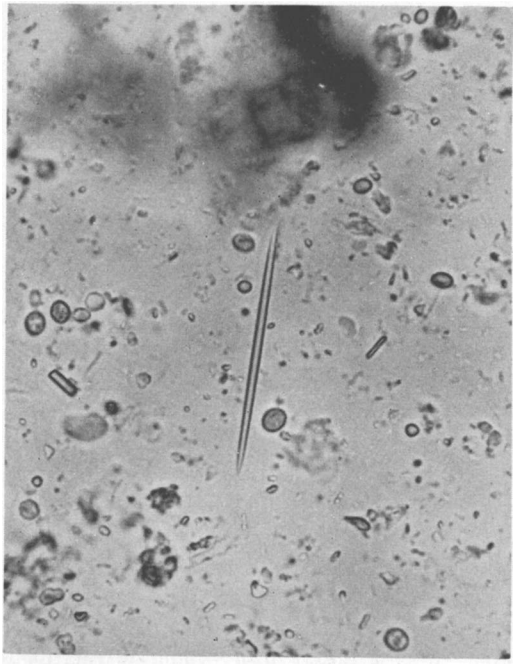

FIG. 6. Crystal frequently found in association with Charcot-Leyden crystals in feces. ×390.

tests and molecular weight studies suggest that the material composing C-L crystals is a polypeptide of low molecular weight containing tyrosine or tryptophane or both. It is soluble at pH 2 and pH 11.2. It was possible to recrystallize a dilute (0.5%) solution of C-L crystals by adjusting the solution to neutrality. No measurable amounts of phosphorus or zinc could be detected by spectrographic analysis.

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Effect of Thiabendazole Upon Experimental Trichinosis in Swine. (27443)

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The anthelmintic property of thiabendazole has been reported recently by Brown *et al.* (1), and Cuckler(2), and the activity of this compound against trichinosis in rats and mice has been reported by Campbell(3). Since subsequent experiments have revealed that large, well-tolerated doses of thiabendazole kill *Trichinella* larvae in the muscle of mice, experiments were carried out to determine the effect of this compound on trichinosis in swine.

Materials and methods. Pigs were exposed to *Trichinella spiralis* larvae recovered by digestion from the ground carcasses of experimentally infected mice and rats. The number of larvae recovered was estimated by a sampling method. In Experiment 1 the larvae were given to the pigs with little or no removal of the digestive solution. In Experiment 2 the larvae were washed by sedimentation in water, before being given to the pigs. The larvae were administered through rubber tubing inserted into the stomach or esophagus. The suspension of larvae was stirred continuously while the inocula were being withdrawn.

In both experiments muscle samples taken

at necropsy were ground twice in a meat grinder and digested in 0.7% pepsin containing 1.0% hydrochloric acid. Sufficient volume was used to allow at least 25 ml digestive fluid for each gram of muscle. The digestion mixture was incubated at about 37°C for 4 to 5 hours, while being continuously agitated. In Exp. 1 replicate samples digested for about 24 hours yielded approximately the same number of larvae as did those digested for 4 to 5 hours. The digest was filtered through gauze, allowed to settle, and adjusted to a volume convenient for counting. The larvae were counted in aliquots varying from 0.075 ml to 1.0 ml, withdrawn while the digest was stirred. If no larva was seen in the aliquots, the suspension was allowed to stand, and the entire sediment was examined. In our experience, larvae recovered by digestion always include occasional larvae which are almost straightened out and evidently dead. In Exp. 1 such larvae were rarely observed and were not counted; in Exp. 2 they were more numerous and were counted separately.

Cross-bred pigs (Hampshire × Yorkshire) were used; they were 12-14 weeks old in Exp. 1, and 5-8 weeks old in Exp. 2. Each experimental group was housed in a separate pen containing a feed hopper to which the pigs had unrestricted access. The pigs and the feed hoppers were weighed every few days.

Experiment 1. Eight pigs were exposed to 73,000 larvae each (2,150-3,320 larvae per kg of body weight). Medication was applied for selected periods, and necropsy was performed on the 26th or 27th days after exposure. Samples were taken from (a) the diaphragm, (b) the basal portion of the tongue, together with both masseters, (c) the abdominal body-wall, (d) the inter-costal muscles, (e) the *longissimus dorsi,* subsequently referred to as "back" muscle, and (f) the muscles of the femoral area, hereafter termed "ham". A 15 g aliquot of each ground sample was digested and counts were made of the larvae recovered. For 1 pig in each group, additional 25 g aliquots of diaphragm and masseter/tongue were digested to test the reliability of the method used; these samples yielded approximately the same number of larvae per gram as did the 15 g samples from the same pigs. *Prophylactic.* Two groups, of 2 pigs each, were fed a diet containing 0.01% and 0.1% thiabendazole, respectively. The medicated diet was administered for one day prior to exposure to *T. spiralis,* and was provided continuously until the 6th day after exposure. *Therapeutic.* Two pigs were fed diet containing 0.15% thiabendazole beginning on the 5th day of infection and ending on the 12th day.

Experiment 2. Twenty-four pigs were divided into 6 groups of 4 pigs each. Twenty pigs were inoculated orally with 140,250 *T. spiralis* larvae each (14,760 to 29,220 larvae per kg of body weight). *Prophylactic.* Two groups of pigs received diet containing 0.01% and 0.1% thiabendazole, respectively, for a period of 9 days, beginning 2 days before exposure to infection. At necropsy, 39 or 40 days after exposure, the entire diaphragm of each pig was removed and ground, as also were large pieces of abdominal muscle and ham. For each pig a composite sample of ground tissue consisting of 25 g diaphragm, 25 g abdominal muscle and 50 g ham was digested. In the smaller pigs 25 g represented almost the whole diaphragm. *Therapeutic.* Two groups of pigs were fed diet containing 0.3% thiabendazole, beginning on the 14th and 28th days after exposure to infection, respectively. In each group medication was continued for 1 week, and the pigs were killed 46 days after exposure. The sampling of muscle and counting of larvae were carried out as in the prophylactic part.

Results. Experiment 1. Prophylactic. The necropsy findings (Table I) indicate that the feeding of 0.01% thiabendazole was ineffective in preventing trichinosis in swine. The diet containing 0.1% thiabendazole provided complete prophylaxis in one pig (No. 618) while the other pig (No. 617) had a barely detectable infection. *Therapeutic.* No evidence for a therapeutic effect was obtained, since there was no significant difference between numbers of larvae recovered from the pigs fed 0.15% thiabendazole and numbers recovered from the control pigs.

Experiment 2. The infection proved to be overwhelming for the smaller pigs, 5 of which succumbed. Two of these (Nos. 762 and

TABLE I. Expt. 1; Prophylactic and Therapeutic Effect of Thiabendazole on a Moderate Infection of *T. spiralis* in Swine.

	Control		Prophylactic* % thiabendazole in feed				Therapeutic†	
	None		.01%		.1%		.15%	
Pig No.:	619	614	615	616	617	618	612	620
Weight—kg								
Initial	22.0	33.0	29.0	27.0	31.0	34.0	34.0	33.0
At 4 weeks	37.0	53.0	45.0	46.0	48.0	55.0	54.0	51.0
Mean daily consumption								
Feed (kg)	1.9		1.8		1.4		1.5	
Drug (mg/kg)	—		6.5		43		58	
Larvae/g muscle from:								
diaphragm	942	161	147	116	<1	0	178	78
jaw and tongue	803	111	106	11	<1	0	117	55
abdominal wall	328	83	65	22	<1	0	115	9
inter-costal spaces	204	37	40	44	<1	0	17	25
back	142	27	5	20	<1	0	27	10
ham	154	50	22	24	0	0	48	5

* Drug fed continuously from one day before exposure until 6th day of infection.
† Drug fed continuously from 5th to 12th day.

764) were in the "therapeutic" groups but died before treatment had been started and are therefore listed in Table II as "infected controls". Three of the pigs, Nos. 762, 800 and 764, died on the 12th, 13th and 15th day, respectively, after exposure, and their intestines were found to harbor approximately 28,000, 77,000 and 53,400 adult *Trichinella*, respectively. The 4th pig, No. 786, was prostrate on the 24th day; it was kept alive by bottle feeding, but continued to lose weight and was killed on the 28th day when moribund. The intestine of this pig yielded approximately 1200 adult *Trichinella*, while a muscle sample was estimated to contain 16,758 larvae per gram. *Prophylactic.* The number of larvae recovered per gram of composite muscle sample is recorded in Table II. None of the pigs receiving 0.01% thiabendazole died, though only one of them (No. 748) made appreciable weight gain during the experiment. At necropsy the 3 smallest pigs had numbers of larvae comparable to the numbers in some of the control pigs, but the heaviest pig had fewer larvae.

To test the infectivity of the larvae recovered from one of the pigs fed 0.01% thiabendazole, 2 mice were each inoculated orally with 975 larvae from Pig No. 743. At necropsy 23 days later, the mice were found to harbor only 270 larvae per gram of carcass. Five mice, each given 1,680 larvae from control Pig No. 786, subsequently yielded 7,710 larvae per gram. It can be concluded that the larvae from the treated pig were infective for mice.

There were no deaths among the pigs receiving 0.1% thiabendazole, and they all gained weight steadily throughout the experiment. At necropsy 40 days after exposure, 3 of the pigs were apparently free of larvae, since none could be demonstrated in the samples of diaphragm, abdominal muscle and ham. The 100 g sample of muscle tissue from the 4th pig (No. 742) yielded 2 larvae per gram.

Therapeutic. One pig (No. 789) had lost 0.7 kg in weight when treatment was started. Within 2 days after treatment had started, this pig was prostrate, and it died on the 22nd day after exposure, 1.5 kg lighter than its initial weight. It is unlikely that this pig ate much of the medicated diet, and 3,192 living larvae per gram were rceovered from the sample of tissues taken *post mortem*. However, an almost equal number of dead larvae (2,574 per gram) was recovered, suggesting that the pig consumed enough thiabendazole to kill a considerable proportion of the larvae. The recovery of so many dead larvae, as compared to the numbers recovered from the other treated pigs, probably resulted from the fact that Pig No. 789 died and was examined at the end of the treatment period, without

TABLE II. Expt. 2; Prophylactic and Therapeutic Effect of Thiabendazole on a Severe Infection of *T. spiralis* in Swine.

Thiabendazole in diet	Mean daily consumption: Kg feed	mg/kg drug	Pig No.	Body wt (kg): at exposure	at 2 wk	at 5 wk	Larvae per g of sample†
None; non-infected controls	1.000	—	732	12.0	16.8	28.3	—
			747	10.0	13.3	24.8	—
			780	7.5	10.0	17.3	—
			790	7.5	10.0	16.8	—
None; infected controls	.575	—	740	6.3	7.8	10.5	1,696(<1)
			750	9.0	11.8	17.0	2,886(<1)
			786	7.3	7.0	(dead)	16,758(<1)*
			800	6.8	(dead)	—	*
			762	5.3	"	—	*
			764	4.8	4.0	(dead)	*
.01%, days −2 to +7	.250	34	741	7.3	7.3	9.0	2,621(<1)
			743	6.0	5.3	6.0	4,808(2)
			748	9.5	12.0	19.8	829(<1)
			779	8.0	7.3	8.3	2,792(<1)
.1% days −2 to +7	.430	55	739	7.8	10.5	19.3	0
			742	8.0	10.5	16.8	2(3)
			787	8.3	11.0	19.0	0
			792	7.3	10.0	18.8	0
.3%, days 14 to 21	.286	86	737	10.0	11.5	19.5	127(34)
			751	9.0	12.3	19.0	119(38)
			789	7.0	6.3	(dead)	5,766(45)*
.3%, days 28 to 35	.457	130	734	10.3	11.8	16.5	103(31)
			783	7.5	7.0	8.5	938(23)
			785	6.5	6.8	7.8	198(44)

* Dead or moribund before scheduled necropsy.
† Percentage of dead larvae given in parentheses.

the usual week's holding period. The surviving pigs (Nos. 737 and 751) yielded very small numbers of larvae at necropsy. It should be noted, however, that these pigs were initially heavier than average, and had made substantial weight gains before treatment. Nonetheless, there is little doubt that the very small yield of larvae from these 2 pigs was the result of chemotherapy.

In the group of pigs fed 0.3% thiabendazole from the 28th to the 35th day, 2 pigs had made very poor weight gains by the time treatment was begun. Although the poor physical condition and small weight gains of 2 of the pigs indicate that their infection was initially very heavy, relatively few larvae were recovered at necropsy from the muscle samples from each pig in the group.

The live larvae recovered from the pigs fed thiabendazole therapeutically were smaller than those from the control pigs. Measurement of several larvae from Pig. No. 737 indicated that they were approximately 25% shorter, and noticeably thinner, than control larvae. Larvae recovered from each of the 6 pigs in the therapeutic trial were fed to mice to test their infectivity. Five mice each inoculated with 1,000 larvae from Pig. No. 789, yielded 6,600 larvae per gram of carcass at necropsy 38 days later. Five groups of 10 mice were inoculated with 200-270 larvae per mouse, from each of the 5 remaining pigs. At necropsy 23 days later the pooled diaphragms from the mice in each of the groups (exposed to larvae from Pig Nos. 737, 751, 734, 783 and 785) yielded 0, 0, 0, 8 and 0 larvae, respectively.

Discussion. Thiabendazole provided a high degree of protection to swine exposed to moderate or severe infections of trichinosis. Unpublished experiments with mice have shown that a diet of 0.1% thiabendazole is fully effective when fed for only one day before exposure to *T. spiralis*, until one day after exposure. Furthermore, thiabendazole removes the worms from the intestines of mice only if administered within one day after infection. These findings suggest that the 7- or 9-day medication period employed in the studies on swine was unnecessarily long, since

the prophylactic effect of thiabendazole appears dependent upon the presence of the drug at time of exposure.

The feeding of thiabendazole to swine also exerted a distinct therapeutic effect. The data in Exp. 2 demonstrate that many *Trichinella* larvae were killed in the muscles of the treated pigs. The number of larvae in the tissues of the pigs fed 0.3% thiabendazole was reduced by more than 80%, as compared to the surviving untreated control pigs. The recorded numbers of dead larvae are perhaps minimal, since their persistence in the host muscle, and their ability to withstand artificial digestion, are uncertain.

Larvae were present in the swine fed 0.15% thiabendazole beginning on the 5th day. Since a proportion of the adult *Trichinella* worms persists in the intestine of swine for several weeks, it is quite probable that in Pig Nos. 612 and 620 some larvae were shed after thiabendazole medication had been discontinued. Such larvae, however, would have had a period of only 2 weeks in which to develop, before necropsy, and therefore might not have survived the digestion procedure (4). Similarly, in Exp. 2, it is conceivable that the larvae recovered from the pigs treated from the 28th to 35th day had been deposited after treatment. In pigs 737 and 751, treated from the 14th to 21st day, the female worms in the intestine at the end of medication might have deposited larvae which would have had about 3 weeks' development before necropsy. The small size and lack of infectivity of larvae recovered from pigs 737 and 751 could thus be attributed either to the direct effect of thiabendazole on the larvae, or to the drug's effect on the reproductive processes of the adults.

Summary. Pigs fed a diet containing 0.1% thiabendazole and exposed to larvae of *T. spiralis* developed either no infection or an infection of negligible severity. A concentration of 0.01% thiabendazole in the diet did not prevent pigs from acquiring trichinosis, but no mortality occurred when the pigs were exposed to huge numbers of *Trichinella* larvae during the period of medication. A moderate experimental infection of trichinosis was not eradicated by feeding 0.15% thiabendazole for 7 days, beginning on 5th day of infection. Pigs fed 0.3% thiabendazole for 7 days, beginning 2 or 4 weeks after exposure to massive infections, suffered no mortality, while 4 of 6 untreated pigs died. The data further indicate that the majority of the *Trichinella* larvae in the muscles of the treated pigs were killed by the medication employed.

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Seasonal Variation in Thyroid Gland Activity in Deermice.* (27444)

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Changes in the morphology, activity and iodine content of the thyroid gland of many species of wild and domestic animals(1-7) have been reported with changes in the season. Heretofore, however, seasonal studies on the thyroid gland of closely related, inter-

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