Table 1. The amounts of patulin, on oven-dry soil basis, required in autoclaved and non-autoclaved media to limit shoot length and weight of Cheyenne wheat seedlings to 50 percent of that of the untreated.

Treatment	Patulin concentration (ppm) required to limit	
	Shoot length	Shoot weight
Sand		
Autoclaved	13	20
Not autoclaved	32	50
Peorian loess		
Autoclaved	37	50
Not autoclaved	120	125
Sharpsburg silty clay loam		
Autoclaved	62	75
Not autoclaved	500	500

phenoxyacetic acid (2,4-D), indoleacetic acid (IAA), and coumarin. With a test substance concentration of 50 parts per million (ppm), germination as compared with untreated seed was as follows: 2,4-D, 40; coumarin, 80; IAA, 85; and patulin, 85 percent. Root growth was reduced to 50 percent of the untreated length by these concentrations: 2,4-D, 1 ppm; coumarin, 9 ppm; IAA, 25 ppm; and patulin, 20 ppm. Shoot growth was reduced to 50 percent of the untreated length by these concentrations: IAA, 63 ppm; coumarin, 20 ppm; patulin, 40 ppm; and 2,4-D, 7.5 ppm.

A test of patulin with Cheyenne wheat coleoptile sections, with and in the absence of small amounts of IAA, revealed that patulin acts as an auxin synergist at concentrations ranging from 0.01 to 10 ppm. Patulin is an unsaturated lactone and its synergistic effect is similar to that of coumarin. Patulin alone at low concentrations shows no growth-promoting effect on wheat coleoptile sections, and at concentrations exceeding 10 ppm it is inhibitory and toxic.

The effect of patulin on Cheyenne wheat seedlings grown in soil autoclaved for 1 hour at 15 lb/in.^{2} steam pressure and in nonsterile soil was tested with sand, Peorian loess, and Sharpsburg silty clay loam. Soil was placed in 9-cm petri dishes supported on No. 8 rubber stoppers, over water in 14-cm petri dishes in which were inverted 1-liter beakers so as to maintain a humid atmosphere. Duplicate dishes containing 15 seeds each were incubated for 5 days at a soil moisture level near saturation and at a temperature averaging about 25°C. After growth for 5 days, the shoots were cut off at the soil surface, measured for length, dried at 65°C, and weighed. The Sharpsburg soil, containing considerable organic matter and, hence, having the greater microbial activity, required larger amounts of patulin to reduce the growth of the plants (Table 1) than Peorian loess, which is low in organic matter. These results are in agreement with those of other investigators (*3*).

Adding patulin to the soil in amounts necessary to suppress plant growth to about 50 percent of normal caused a rapid development of a new microbial population. Many colonies of mold appeared on the surface of the Sharpsburg soil. Isolates of these colonies grown in potato dextrose broth developed substances toxic to corn seedlings. Microscopic examination showed the isolates did not belong to the *Penicillia*. Thus, although soils with the larger amounts of organic matter have a greater ability to neutralize the phytotoxic effect of patulin, they may have the potential to develop altered microbial populations that may produce substances strongly toxic to germinating seeds.

The patulin treatments in autoclaved and nonsterile soil showed root growth inhibition but not root curling and twisting that was observed with tests of the culture fluid and in the tests when *Penicillium urticae* Bainer was inoculated into sterile amended soil. This organism evidently produces another substance besides patulin that has an effect on root growth and causes root curling.

Patulin is produced by a number of fungi. Its effect on other fungi and, hence, indirect effect on crop plants may be significant. Stubble-mulch farming systems may provide ecological conditions such as concentration of organic matter and environmental relationships, especially in years of normal to above-normal rainfall, favorable for the growth of microorganisms producing phytotoxic substances.

Patulin production under field conditions and subsequent effect on field crops remain to be ascertained (*4*).

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Schistosoma mansoni: Development of Challenge Infections in Mice Exposed to Irradiated Cercariae

Abstract. *The principal effect of x-irradiated* Schistosoma mansoni *cercariae may be to slow the migration and development of the worms of a challenge infection. This would account for the smaller number of worms found in the portal system in the early weeks and the delayed accumulation of recoverable worms and eggs.*

When mice, rats or rhesus monkeys are exposed to irradiated cercariae of *Schistosoma mansoni* or *Schistosoma japonicum* and are later challenged with normal cercariae of the same species, they have fewer worms in the portal system, or fewer eggs in the feces or liver in the first 28 to 63 days (*1–3*). It has been concluded from these studies that the animals develop immunity as a result of aborted infections with the irradiated schistosomes. Generally, the difference in worm counts between "protected" and "unprotected" animals has been of the order of about 1 to 2, and Smithers (*2*) has emphasized the incompleteness of the protection afforded. However, in experiments with mice, in which the interval between challenge and examination was only 28 days, larger differences were seen.

Our experiments have produced additional information concerning the manner in which the changes induced by the irradiated cercariae affect the challenge infection.

Schistosoma mansoni obtained from Puerto Rico and maintained in a strain of snails from the same endemic area were used in female white Swiss mice that weighed 17 to 22 g at the start of the experiments. The mice were all ex-

Table 1. Worms and eggs of *Schistosoma mansoni* recovered from mice exposed to irradiated cercariae and challenged 51 days later with normal cercariae. Each figure represents worms or eggs from five mice unless a different number of mice is given in parentheses. Controls received only the challenge infection.

Radiation dose (r)	Number found at various times after challenge			
	33 days	48 days	60 days	103 days
	Worms per mouse			
0	28	17	*	*
3,000	26	28	53	*
6,000	13	16	27	25(3)
9,000	11	15	23	19(2)
12,000	11	17	24	19(1)
15,000	6	17	27	18
Control	25	30	39	28(3)
	Eggs per gram of liver			
0	10,293	8,306	*	*
3,000	0	1,361	13,725	18,313(4)†
6,000	0	985	6,000	15,457(3)
9,000	0	1,167	6,846	13,255(2)
12,000	0	826	4,875	17,920(3)
15,000	0	667	7,790	11,078
Control	0	2,000	10,416	14,256(3)

* No survivors in the group of five mice.
† Mice dead, but livers could be examined for eggs.

posed by the tail immersion method (*4*) after they had been immobilized by intraperitoneal administration of Nembutal. Each mouse received 130 cercariae in the primary and challenge infections. The irradiation was delivered to freshly emerged cercariae by a 2.0- to 2.5-Mev Van de Graaf generator, and exposure times were determined by a built-in automatic timer. Dosages were measured with a standardized ionization chamber. All mice were examined for worms by a standardized perfusion method based on the method of Yolles *et al.* (*5*) after they had received an overdose of Nembutal, which causes the worms to release their hold on the vessels. Liver egg counts were obtained by weighing the livers, treating them in 4 percent KOH overnight at 37°C, and counting eggs from portions of the resulting suspension in Sedgewick-Rafter chambers.

The behavior of cercariae exposed to low radiation doses (ranging from 500 to 1500 r) was not different from that of nonirradiated cercariae both in vitro and in vivo. The worm counts from mice that had received these cercariae and the number of eggs in the liver were not different from those in mice exposed to nonirradiated cercariae.

Some difference occurred with cercariae exposed to higher radiation levels ranging from 3000 to 16,000 r. While their behavior in vitro was not different from that of control cercariae during the first 8 hours after irradiation, during the next 16 hours the irradiated cercariae died in greater numbers than the controls, and the proportion dying increased as the amount of irradiation was increased.

The water in the tubes used for the tail exposures was examined for cercariae after the exposure period to determine whether the irradiated cercariae had penetrated successfully. There was no significant difference in the number of residual cercariae in the test and control tubes. Approximately 25 percent of the cercariae remained in both.

The pattern of challenge experiments designed to test for immunity was as follows: Mice were exposed to cercariae of the same pool immediately after the cercariae had received various amounts of irradiation. At intervals after exposure to these cercariae the mice were challenged with normal cercariae; then they were examined 33 to 103 days later. Evidence of immunity was sought in worm counts and liver egg counts which could be compared with data from suitable control mice.

Data from a typical experiment are presented in Table 1. When the mice were examined 33 and 48 days after challenge the worm counts from mice that had received irradiated cercariae before challenge were much lower than the worm counts from the controls, except for those exposed to cercariae irradiated with the lowest dose (3000 r). However, the counts rose steadily with the passage of time, and after 60 and 103 days the counts were not significantly different from those of control mice. The numbers of eggs in the liver of mice that received irradiated cercariae were also lower than those from control mice at first, but these counts also rose as the challenge infections became older. After 103 days the egg counts from the mice that had received irradiated cercariae were not different from those in mice exposed to cercariae for the first time. In a similar experiment, the challenge was delayed until 113 days after the irradiated cercariae had penetrated the mice, but the results were not substantially different. Ninety days after the challenge the worm counts were essentially the same in both test and control groups, and the liver egg counts were essentially the same in all groups.

It is noteworthy that the eggs recovered from the liver and the worms recovered from the portal system of the test mice were the result of the challenge infection only. Neither eggs nor worms were recovered from mice that had received, 27 to 103 days earlier, cercariae irradiated with doses from 3000 r upward.

It is clear from these experiments and from the reports of others that prior exposure of animals to irradiated schistosome cercariae affects the course of the challenge infection. Thus, within the first weeks after challenge the number of worms recoverable from the portal system and the number of eggs in the liver or feces is smaller if the animals receive prior infection with irradiated cercariae. It has been assumed by some, on the basis of this evidence, that worms of the challenge infection are substantially reduced in numbers due to the induced immunity. Our experience, however, indicates that in mice, at least, this may not be the case. Although low worm and egg counts were obtained in the early weeks after challenge, both worm counts and egg counts rose later to levels not significantly different from those of control animals. It appears, then, that the principal effect of the irradiated worms, which die in the mice before reaching maturity, may be to slow the migration and development of the worms of the challenge infection. This would account for the smaller number of worms found in the portal system in the early weeks and the delayed accumulation of recoverable worms and eggs. Migration of *S. mansoni* in mice is much slower than the migration of *S. japonicum* and *Schistosomatium douthitti* in the same host (*6*) and the effect of the irradiated cercariae seems to be to slow it still more. The possibility that extremely large numbers of irradiated cercariae may partially protect mice against an otherwise lethal exposure to normal cercariae (*3*) must be checked by experiments involving longer observation times.

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