

MYCOTOXIC EFFECT ON FETAL DEVELOPMENT: PINE NEEDLE ABORTION IN MICE

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Chow, Hamar & Udall (1972) reported that a water-soluble ('aqueous') fraction extracted from the needles of yellow pine (*Pinus ponderosa*) could disrupt fetal development of mice and some constituent of the pine needles was believed to be responsible for the activity. However, the aqueous fractions of pine needles harvested in the following 2 years induced little reproductive failure in mice. This inconsistency in activity raised doubts concerning the toxicity of the pine needles.

A preliminary study was undertaken to determine whether metabolites produced by fungi which we had observed on the pine needles could be the cause of the reproductive failure. Several pine needles were placed in the aqueous fraction. Fungi started to grow within 2 days of incubation at room temperature. After 2 weeks of growth, the preparation was filtered through three layers of Whatman No. 2 filter paper and then a Millipore membrane of 5- μ m pore size. The filtrate, designated 'aqueous-fungi', was freeze-dried and mixed with Purina Mouse Chow. The filtrate was fed freely throughout pregnancy, beginning on the first day after mating, which was indicated by the presence of vaginal plugs. Eight-to-twelve-week-old virgin mice of the CFW strain from Carworth were used. The 'aqueous-fungi' preparation showed a significantly greater disruptive effect on pregnancy than did the aqueous fraction. The results of these tests are presented in Table 1. They show that the detrimental effect on pregnancy was increased when the intake of the materials being tested was increased. In less severe cases, mice carried their pregnancies to term, but the young mice were either dead after birth or weak and small. In more severe cases, the fetuses were resorbed. Apparently, the fungi converted some constituents of pine needles into compounds which are toxic to fetal development. There was no evidence of any toxic effect on the mothers.

In order to substantiate the hypothesis that mycotoxins instead of pine needles were the cause of reproductive failure, another experiment was undertaken. The aqueous fraction was autoclaved at 110°C for 15 min to inactivate the toxic agents (Chow *et al.*, 1972). This inactivated aqueous fraction was then used as the medium for the growth of fungi isolated from pine needles. After 2 weeks of fungal growth, the filtrate was prepared following the procedure already described. This freeze-dried preparation of the filtrate was designated 'reactivated aqueous-fungi'. The effects of the inactivated and reactivated preparations on fetal growth were tested on pregnant mice. The results are

shown in Table 2. For this part of the study, the pregnant mice were given the test feeds for 7 days starting on the 1st day of mating. On the 7th day, the mice were killed and the uteri, including the placentae, were isolated and weighed. The weight varied from 400 to 600 mg, depending on the litter size. In the mice fed the 'aqueous-fungi' and the 'reactivated aqueous-fungi', fetal resorption occurred while fetal development in all but one mouse fed the inactivated 'aqueous-fungi' preparations was normal. Evidently, the isolated fungi grown in the inactivated aqueous medium regenerated the activity which affected normal pregnancy.

Table 1. Effect of fungal growth in the aqueous fraction of pine needles on pregnancy in mice

Test ration	No. of mice pregnant	No. of mice giving birth	Av. litter size	Total no. of young mice dead	Av. wt of 1-day-old mouse (g)
Control (Purina Mouse Chow)	8	8	10.6	0	1.55
Aqueous (1:1)*	9	9	10.6	2	1.46
Aqueous (1.5:1)	7	5	8.6	1	1.39
Aqueous-fungi (1:1)	8	3		All	
Aqueous-fungi (1.5:1)	8	1		All	

* Amount of test ration equivalent to 1 or 1.5 g pine needles added to 1 g Purina Mouse Chow. The test ration was fed freely from the first day after mating and throughout pregnancy.

Table 2. Effect of pine needle fungi on the toxicity of the aqueous fraction in pregnant mice

Test ration	No. of mice pregnant	Av. litter size	Av. wt (mg) of uterus and placenta*	No. of mice with resorbing fetuses
Control (Purina Mouse Chow)	10	15.4 (10)†	501 (10)	0
Aqueous-fungi (1.5:1)	8	—	133 (8)	8
Autoclaved aqueous-fungi (1.5:1)	6	12.0 (5)	395 (5)	1
			80 (1)	
Reactivated aqueous-fungi (1.5:1)	8	15.3 (4)	383 (4)	4
			80 (4)	

* Average weight of uterus and placenta after 7 days' gestation.

† Number of observations.

Reproducible results have been obtained with different preparations of 'aqueous-fungi', using the same fungal culture. We have also found a higher activity of the 'aqueous-fungi' preparations with more abundant fungal growth in the aqueous fraction. At present, our results suggest that mycotoxins instead of pine needles may be the factors which cause reproductive failure.

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REFERENCE

CHOW, F. H. C., HAMAR, D. W. & UDALL, R. H. (1972) Reproductive failure of mice caused by pine needle ingestion. *J. Reprod. Fert.* **30**, 169.