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Failure of Blighted Russet Burbank Potatoes to Produce Congenital Deformities in Rats (38088)

RICHARD F. KEELER, DEXTER R. DOUGLAS, AND GILBERT F. STALLKNECHT

U.S. Department of Agriculture, Agricultural Research Service, Western Region, Poisonous Plant Research Laboratory, 1150 East 14th North, Logan, Utah 84321, and Idaho State Branch Experiment Station, Aberdeen, Idaho 83210

Renwick (1) suggested a relationship between maternal consumption of potatoes and fetal anencephaly and spina bifida (ASB) in humans. He found a close relationship between the occurrence of ASB in geographic areas of the world and potato late blight due to *Phytophthora infestans* and suggested that the tuber or the fungus, or both, might somehow be involved. Among the possible teratogens he suggested were the coumarins synthesized by the fungus itself, various endogenous tuber compounds such as steroidal alkaloids, and the terpene phytoalexins synthesized by the tubers in response to the infection.

In the United States late blight due to *P. infestans* occurs principally in the northeastern and north central states, where environmental conditions allow it to thrive, and principally involves potato varieties other than Russet Burbank. However, approximately 40% of the United States potato crop is produced in the Pacific Northwest, an area of low natural ASB incidence (1). The predominant variety grown there is the Russet Burbank. *P. infestans,* the cause of late blight, is not a disease of this area. There are, however, several different pathogenic tuber infections that complicate production. Early tuber blight, caused by *Alternaria solani,* is probably the most severe of these infections. Research at the University of Idaho Aberdeen Branch Experiment Station has shown that phytoalexins common to *P. infestans* infection are also produced as a result of *A. solani* infection (2).

Consequently, we decided to investigate the phytoalexin–teratogen possibility by using *A. solani*-infected Russet Burbank tubers, and the results are reported here. We are investigating other aspects of the problem related to coumarins and alkaloids also, and those results will be reported elsewhere. Rats were fed either lyophilized infected tuber tissue or a chloroform:acetic acid:water fraction of the infected tuber tissue containing the terpenoid phytoalexins. Russet Burbank tubers infected with *P. infestans* were also included for comparative evaluation. We selected the rat for assay for initial studies although neither Poswillo (3) nor Chaube (4) and their coworkers found ASB in offspring of rats fed various potato blight preparations. We did so (a) because we have found rat embryos to be susceptible to the teratogen cyclopamine, an alkaloid of structural similarity to certain potato alkaloids and which produces cyclopia and related cephalic malformations (5), and (b) because one can gavage large quantities into rats—much larger than the quantities given rats by either Poswillo (3) and Chaube (4). Our results of those experiments are reported here.

Materials and Methods. Extracts and infected tubers were prepared as follows. Russet Burbank potatoes (170–280 g) were washed and nine inoculation sites of approximate equidistance randomly selected on each tuber. A plug 10 cm deep was removed from each site with a #4 cork borer. One drop of a spore suspension containing 1800 spores/ml of *A. solani* (a pathogenic

isolate from Russet Burbank) was placed in the bottom of the well created by removal of the plug. The plug was replaced causing the spore suspension to become distributed around the wall as well as at the bottom of the well. Clear petroleum jelly was placed over the plug to keep the cut surface from drying until infection was established. The tubers were incubated at 20°C at 90% relative humidity for 30 days, at which time the plugs were removed and discarded. Cylinders were cut parallel to the well with a cork borer large enough to remove the well plus about 4 MM of healthy tuber tissue surrounding the visible radiating infection. The tissue was frozen with liquid nitrogen, freeze dried, ground in a Wyler[1] mill using a #40 screen, and stored in airtight containers until used.

Tubers to be infected with *P. infestans* were washed and then swabbed with a zoospore suspension of that organism (an isolate from Kennebec potatoes unidentified as to race) and incubated at 20°C at 90% relative humidity for 30 days. Each tuber was hand sliced, and visibly infected tissue, which appeared mottled, and about 4 cm of healthy tissue were harvested and freeze dried. Remaining tissue was discarded. The extracts from tuber tissue were prepared according to Shih, Kuć, and Williams (6) and contained terpenoid phytoalexins.

Pregnant Simonsen[1] Albino rats (Sprague–Dawley derived) were gavaged in the morning with 4 g and again in the evening with 4 g of the freeze-dried *A. solani*- or *P. infestans*-infected tuber preparations per rat on each of the designated days of gestation (see Table I). Others were gavaged once each day on the designated days with the *A. solani*-infected tuber extract containing the equivalent of 11 g of infected fresh-weight tissue. Days 6–9 is the susceptible period in rats for cyclopia and related cephalic malformations (5), whereas days 8–11 is the susceptible period for ASB (7). The rats weighed 250 ± 10 g at Day 6 of gestation. Day 1 of gestation was considered to begin at midnight preceding the finding of sperm in the vaginal tract on morning examination. All rats were provided with Purina rat diet and water *ad lib.* At Day 19 each pregnant rat was killed, implantation sites were counted to assess resorptions, and fetuses were examined grossly for ASB and for cyclopia and related cephalic malformations.

Results. Over 500 offspring (Table I) were found at necropsy in pregnant rats gavaged with the experimental preparations. None had ASB nor cyclopia or related cephalic malformations. Resorptions occurred at higher incidence in the 8–11-day dosing period when each of the experimental preparations was used than in other dosing periods or in control undosed rats. Resorptions often suggest that terata would be produced at lower doses or at the given doses in survivors (8), but this seems to be ruled out here because over 90% survived in this fairly large sampling and were not deformed. The LD_{50} dose for the extracts proved to be about 50% higher than dose levels given. The doses of the freeze-dried material used approached the maximum gavageable quantity and with the *A. solani* preparations caused significant weight loss (Table I) in dams from time period groups selected for weight trials.

Discussion. Because we used only the infected and adjacent part of each tuber (about 20%) in our freeze-dried preparations, whereas both Poswillo (3) and Chaube (4) apparently used whole tubers, we believe our material could have been, on that basis, up to 5 times higher in concentration of any alleged potato teratogen synthesized in response to the infection. Further, we gavaged about 32 g/kg/day of freeze-dried material into the rats, whereas Chaube (3) gavaged 2.5 g/kg/day of fresh-weight homogenate, and Poswillo (4) provided *ad lib.* in a pellet about 2.5 g/kg/day of freeze-dried homogenate mixed with rat diet. We can make a correction for the weight difference and for the 80% moisture content of the tuber (Chaube experiment), and can make a correction for the weight difference and the assumed 50% feed con-

[1] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

TABLE I. Effects of Experimental Preparations on Rat Offspring.

	Number of animals	Live offspring	Resorptions	Normals	Abnormals	Average weight change[a] (g)
Controls	8	82	0	82	0	+7
Freeze-dried *A. solani* preparation						
6– 9 days	6	61	1	61	0	−9
7–10 days	5	48	0	48	0	
8–11 days	8	94	5	94	0	
Freeze-dried *P. infestans* preparation						
6– 9 days	6	62	1	62	0	+15
7–10 days	4	43	4	43	0	
8–11 days	10	103	10	103	0	
A. solani extract						
6– 9 days	2	19	0	19	0	−6
7–10 days	2	22	1	22	0	
8–11 days	9	89	6	89	0	

[a] Change in weight of dam from Day 5 to Day 8 of gestation.

sumption (Poswillo experiment). The calculations suggest we may have gavaged doses that were higher an additional 65 times compared to the former or 26 times compared to the latter. Overall our doses may have been, therefore, some 100–300 times higher in alleged teratogen compared with doses gavaged by these workers.

The freeze-dried infected and adjacent tuber material gavaged in our rats would be equivalent on a weight basis to a daily consumption of nearly 2 kg of this infected material or 10 kg of whole tuber in a 60-kg human. Because visibly diseased parts of the tuber are usually removed before cooking and eating, considerably more than 10 kg (possibly 20–30 kg) of disease-free material would need to be eaten by a human to equal our rat doses.

Summary. Pregnant rats were gavaged with massive doses of freeze-dried late blight infected Russet Burbank tuber tissue, freeze-dried early blight infected tuber tissue, or an extract of the latter containing terpenoid phytoalexins. There were no grossly detected deformities observed in over 500 offspring. The results suggest that neither early nor late blight infected Russet Burbank potatoes are teratogenic in rats at massive doses and, by extension, that the terpenoid phytoalexins likely are not either.

1. Renwick, J. H., Brit. J. Prev. Soc. Med. **26**, 67 (1972).

2. Stallknecht, G. F., and Douglas, D. R., Phytopathology **63**, International Congress Abstracts (1973).

3. Poswillo, D. E., Sopher, D., and Michell, S., Nature **239**, 462 (1972).

4. Chaube, S., Swinyard, C. A., and Daines, R. H., Lancet **i**, 329 (1973).

5. Keeler, R. F., Lancet **i**, 1187 (1973).

6. Shih, M., Kuć, J., and Williams, E. B., Phytopathology **63**, 821 (1973).

7. Warkany, J., "Congenital Malformations," pp. 193–195, 275. Year Book Medical Publishers, Inc., Chicago (1971).

8. Fraser, F. C., and Fainstat, T. D., Pediatrics **8**, 527 (1951).

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