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Robert C. Goss
State College of Iowa

Robert Stevens
State College of Iowa

Experimentally Induced Mammary Tumors in Swiss Mice with *Cephalobus* (Nematoda Cephalobidae)[1]

ROBERT C. GOSS[2] AND ROBERT STEVENS[3]

Abstract. Mammary tumors developed when 7-month-old Swiss-Webster female mice were subcutaneously injected with nematodes in the dorsal frontal area of mammary tissue. Reactions observed were loss of vibrissae (47%), development of tumors (24%) and death (10%). Loss of vibrissae occurred within 1 or 2 weeks after the third injection. Tumors developed between the seventh and tenth week. No animal developed multiple tumors involving different regions of the body nor did spontaneous tumor regression occur.

Nematodes tentatively assigned to the genus *Panagrodontus* were isolated from the tubes of the pitcher plant *Sarracenia sledgei* Macfarlane, in southwest Louisiana (4). The data indicated that the nematode used the plant as a normal habitat. The presence of plant enzymes complicates the problem of interdependence between the plant, microbes, digested captured fauna and nematodes.

The above observations lead to the speculation that the nematode has anti-enzyme systems which block the native enzymes of the plant or has a substance which is secreted and counteracts the host's enzymes. It was felt that this phenomenon might be relative to the cancer problem if the working hypothesis accepted was that the nematode had altered, through an unknown mechanism, the enzyme profile of the pitcher plant. It is we'l known that normal cells can be transformed into cancer cells experimentally by a variety of agents diverse in nature and properties (2). The diversity of these agents suggests that they induce cancer by altering, through different pathways, the same cellular function.

Credit for the first description of mammary tumors caused by nematodes is given to Borrel (1). He described a nematode frequently found in mouse tissues, which he believed might convey the actual cancer organism. Fibiger (3) was able to induce numerous cancers in the fore-stomach of rats by feeding them a nemotode isolated from the muscles of a cockroach. He described these neoplasms as possessing exactly the same histological structure as epitheliomata in man and animals.

[1] Supported by the Iowa Division of the American Cancer Society.
[2] Assistant Professor, State College of Iowa, Cedar Falls.
[3] Undergraduate assistant, State College of Iowa, Cedar Falls .

The purpose of this paper is to present evidence that females of the Swiss-Webster strain of mice will develop neoplasms (carcinomas) under experimental conditions when injected with nematodes of the genus *Cephalobus*. *Cephalobus* belongs to the same superfamily as the nematode isolated from the pitcher plant. *Cephalobus* was used because of the ease of handling and culturing.

Procedure

Cultures of *Cephalobus* were obtained from soil samples in South Carolina and maintained on sterile potato plugs in test tubes. A screening program was initiated to find a substrate which would support the nematodes with a minimum of microbial and chemical contamination. Preliminary screening narrowed the number acceptable to three. They were commercial Pablum, oatmeal, and Flav-R-Pac instant potato mix. The potato mixture was selected because of the low protein content.

Ten grams of instant potato mix and a 9 cm filter pad were placed in standard petri plates and sterilized. After the mix had cooled, nematodes suspended in 15 ml of 10% sodium propionate (w/v) were added to the petri plates. The plates were incubated under standard room conditions.

The nematodes were harvested from the top of the plate by washing with distilled water, then were placed in a sterile Seitz filter with a 5 to 6-micron asbestos pad. They were washed, under aseptic conditions, with 500 ml of sterile distilled water. This was followed by two or three washes with the following chemicals: 10% sodium propionate, pH 4.0 buffer, 0.03% hydrogen peroxide, pH 7.0 buffer (phosphate), 1% penicillin, and 1% streptomycin. The nematodes were allowed to remain in each solution for approximately 10 minutes before the filter was flushed with 500 ml of sterile distilled water. The filter pad was removed under aseptic conditions and placed in a sterile petri plate. Sufficient sterile buffer (pH 7.0) was added to free the nematodes from the filter pad. By use of sterile disposable Pasteur pipettes equipped with 30 ml rubber bulbs, the nematodes were transferred to sterile vaccine bottles.

The experimental animals were 7 months old, and from two to eight and were kept in each of several mice cages under standard laboratory conditions of temperature, humidity, and daily fluctuations of light. As standard procedures the animals were housed under laboratory conditions for 3-4 weeks or longer before the injection schedule began.

Usual clinical procedures were followed in injecting the animals subcutaneously in the dorsal frontal area of mammary

tissue. The calculated dosage was injected at weekly intervals followed by a second injection 11-14 days later.

Discussion

A constant problem in the nematode cultivation was contamination by *Rhizopus nigricans*. Visible bacterial or yeast colonies on or in the medium rarely occurred. Direct exposure of nematodes to a 10% solution of sodium propionate for 30 minutes resulted in an average death rate of 16%. A solution of 5% to 10% (w/v) appear to be the maximum tolerated and the best level for inhibition of fungi. Use of a 15% solution decreased usable plates by 26% and the number of viable nematodes by 8%.

Microbial isolation (Difco PDA and nutrient agar) indicated that the washed nematodes were contaminated with a large Gram-positive sporulating rod. Unsuccessful attempts were made to isolate the bacterium from the injection site at various intervals up to 14 days after injection. A series of animals were injected with the final wash solution. No reactions were observed. This would indicate that the bacterium was a saprophyte and that the animal's defense reaction was adequate.

In the experiments pathogenicity was interpreted to mean that the nematode, after being injected into the mouse, would cause death or other readily recognizable symptoms. Toxin production could be an important feature of the virulence of the organism or agent. The mouse is generally less susceptible to exotoxins but is particularly susceptible to endotoxins.

Approximately 85% of the animals reacted, either by loss of vibrissae, developing tumors, or death, when injected with the nematode (Table 1). Loss of vibrissae occurred within 1 or 2 weeks after the second or third injection. Tumors developed between the seventh and tenth week and continued until the twenty-third week at which time the animals were killed.

Table 1. Symptoms observed in Swiss strain females after injection with *Cephalobus* sp. Experiment terminated 23 weeks after second injection.

Number of mice	Injection		Symptoms observed		
	Primary	Second§	Loss of vibrissae	Development of Tumor	Death
10	0.3 ml	0.0 ml	3	2	0
10	0.6	0.0	6	2	0
10	0.9	0.0	6	3	3
10	0.1	0.1	4	2	2
10	0.2	0.1	5	0	5
10	0.3	0.3	3	6	0
10	1.0	0.6	6	2	0
22	0.0	0.0	0	0	3

§ Second injection is 11-14 days after last primary injection.

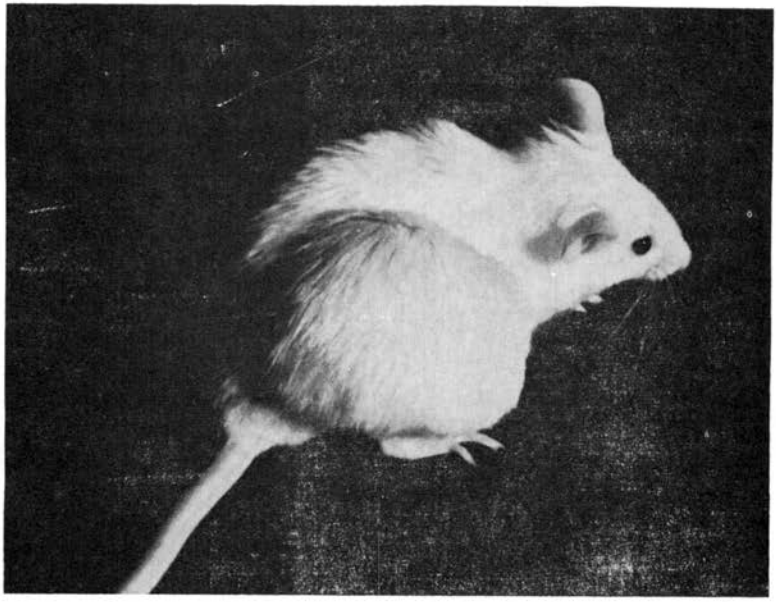

Figure 1. Appearance of mammary tumor in Swiss strain female 16 weeks after last injection with 0.9 ml *Cephalobus* sp.

Loss of vibrissae would interfere with the animal's orientation to its environment with respect to gravity and acceleration. This in turn would confuse the animal in its eqvilibrium. No effects were observed. Evidently the vibrissae were lost gradually and the animal was able to adapt.

When the tumors were small but grossly detectable the animals began to develop progressive signs of general ill health, evidence of malnutrition, and in the late stages a general unthrifty appearance of the hair. About 40% of the animals had a shrunken appearance which would indicate kyphosis. The animals with the larger tumors (Figures 1 and 2) were greatly inconvenienced by the neoplasm. In some instances the tumor interfered with movements and feeding. Spontaneous tumor regression did not occur during the experiment.

Complete autopsies were routinely performed and tissues were saved from all body regions offering any suggestion of abnormal growth. Blood and tissue samples were collected from various body cavities and microscopically examined for nematodes or nematode debris. Slight liver toxicity was observed in a small number of animals. Nematodes and debris were not observed at the injection sight nor in cytopathological preparations of tumor tissue. No animal developed multiple tumors involving different regions of the body nor did spontaneous tumors appear

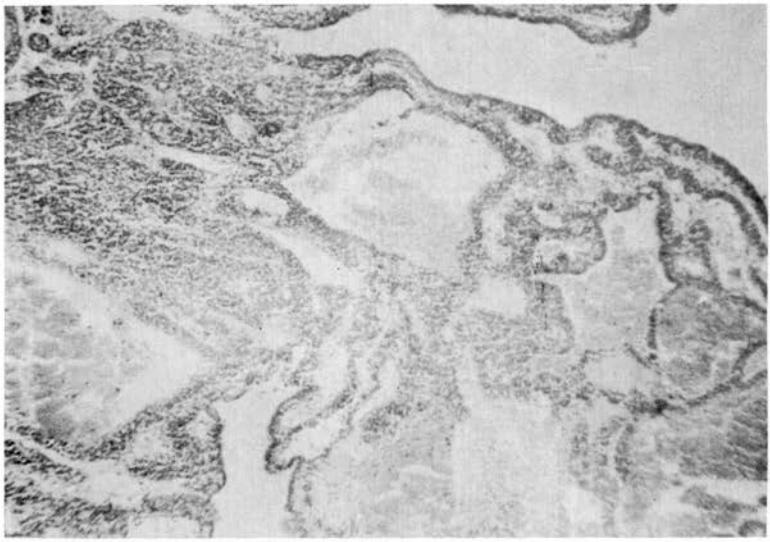

Figure 2. Cytopathological appearance of the cords and masses of epithelial cells in the adenocarinoma of the mouse mammary gland.

in the controls. Death of the animals could be attributed to secondary infections or to age.

The data indicate one of the following conclusions: (1) there is a virus-nematode association. The literature in phytopathology indicates that certain soil nematodes are carriers of plant viruses; (2) the ability of the nematode to enter the cell and alter its genetic mechanism is probably dependent upon factors other than the mere presence of the nematode. In the development of crown gall of plants, for example, cellular changes induced by exposure to carcinogens do not take place unless the pith is in a growing phase (5); (3) the observations to date are simply an interesting phenomenon which can be explained on the basis of the biological variation in non-inbred Swiss mice. The acceptability of the third conclusion would be based on a screening program using in-bred strains of mice very susceptible to mammary tumors.

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