genus since the larvae of the related legume-feeding *E. varivestis* frequently feed in groups. Although a leaf may have several old feeding scars, no leaves with multiple fresh scars were seen. When a leaf is damaged, the wound-induced substance may increase in the entire leaf. We have no direct evidence that this happens but extensive responses to damage are known. Green and Ryan (*11*) found an increase in proteinase inhibitors in damaged tomato leaves. After several hours, levels of proteinase inhibitors increased in much of the undamaged parts of the plant as well. Ryan and others (*12*) have subsequently described the widespread occurrence of mobilized proteinase inhibitors in many plants.

We found long-distance movements after single morning feeding periods (Table 3). Between two successive feeding periods (two morning periods) the beetles traveled an average of 6 m (*13*), but the much less mobile larvae move as well. Larvae were never feeding any closer than 2 m from fresh feeding scars. It is difficult to understand why behavior for such frequent movements should be favored unless such behavior results in avoiding areas where levels of chemical feeding deterrents are increasing. It is unlikely that parasitoid or predator avoidance is involved since these reflexively bleeding, brightly colored, conspicuous insects are not likely to counter predation by moving.

The phenomenon described for *E. tredecimnotata* may be common. For example, the larvae of monarch butterflies occasionally cut into the leaf petiole before feeding on the leaf (*14*). Adult milkweed cerambycids often chew into the stem tissue above their egg deposition site (*14*). *Heterocampa* larvae often cut into the petioles of sugar maple leaves before feeding on the leaf (*14*). This curious behavior may perhaps be explained as an adaptive response to rapidly mobilized plant defenses. More generally, the frequent observation that herbivores often move before the food supply is exhausted may be a common method to avoid a local accumulation of chemical defenses. Thus, wasteful feeding may be adaptive. Of agricultural significance is the possibility that such frequent movements to avoid local increases in chemical deterrents may increase the spread of pathogens that are carried by herbivore vectors.

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References and Notes

1. D. A. Levin, *Annu. Rev. Ecol. Syst.* **7**, 121 (1976).
2. W. E. Wallner and G. S. Walton, *Ann. Entomol. Soc. Am.* **72**, 62 (1979).
3. U. Röttger and F. Klingauf, *Z. Angew. Entomol.* **82**, 220 (1976).
4. E. Haukiója and P. Niemelä, *Ann. Zool. Fenn.* **14**, 48 (1977).
5. D. F. Rhoades, in *Herbivores: Their Interaction with Secondary Plant Metabolites*, G. A. Rosenthal and D. H. Janzen, Eds. (Academic Press, New York, 1979), p. 29.
6. This study was located on the grounds of the Colegio Superior de Agricultura Tropicál, 27 km northeast of the town of Cárdenas in Tabasco, Mexico.
7. C. P. Da Costa and C. M. Jones, *Hort. Sci.* **6**, 340 (1971).
8. O. L. Chambliss and C. M. Jones, *ibid.* **89**, 394 (1966); F. Gould, thesis, State University of New York, Stony Brook (1977).
9. O. L. Chambliss and C. M. Jones, *Science* **153**, 1392 (1966); W. L. Howe and A. M. Rhodes, *Environ. Entomol.* **5**, 747 (1976); W. L. Howe, J. R. Sanborn, A. M. Rhodes, *ibid.*, p. 1043; S. Risch, personal communication.
10. G. C. Sharma and C. V. Hall, *Environ. Entomol.* **2**, 154 (1973).
11. T. R. Green and C. A. Ryan, *Science* **175**, 776 (1972).
12. C. A. Ryan, *Plant Physiol.* **54**, 328 (1974); D. McFarland and C. A. Ryan, *ibid.*, p. 706; M. Walker-Simmons and C. A. Ryan, *ibid.* **59**, 437 (1977); *ibid.* **60**, 61 (1977); *Biochem. Biophys. Res. Commun.* **74**, 411 (1977).
13. Beetles and the leaf on which they were observed feeding were marked. Each of 36 beetles was marked with a small dot of Testors nontoxic paint on the right elytra, and a number was written on the dot. Marked beetles were captured in the morning near the end of the feeding period. After being marked, the beetles were left individually in gauze bags for the paint to dry. After approximately 30 minutes, the bags were opened beneath the leaf on which they had been feeding. Invariably, the beetles crawled onto the lower surface of the leaf and showed no alarm behavior. Over 20 days, beetles were recaptured and their new feeding site was marked. Distances between successive feeding sites were measured. The average distance between nearest-neighbor squash leaves was approximately 0.25 m. Of the 36 marked beetles, only 9 were recaptured (Table 3).
14. C. Carroll, unpublished observation.
15. We thank S. Risch, Cornell University, and the faculty and students of the Colegio Superior de Agricultura Tropicál for their encouragement. This is contribution 355 from the Program of Ecology and Evolution at the State University of New York, Stony Brook.

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Tumor Anorexia: A Learned Food Aversion?

Abstract. *Anorexia can occur when a specific diet is associated with a developing illness. The studies reported here show that the decline in food intake which accompanies tumor growth is accompanied by the development of aversions to the specific diet consumed during tumor growth. An immediate elevation in food consumption occurred when a novel diet was introduced. Therefore, the development of learned aversions to the specific diet eaten during tumor growth may be a causal factor in the development of tumor anorexia.*

Reduced appetite and subsequent weight loss are often seen in patients with neoplastic disease (*1*). Although a number of possible mechanisms for this anorexia have been suggested, agreement about the actual causes of tumor-produced loss of appetite and weight has not been reached (*2*). One possible explanation for anorexia in patients receiving treatments with severe and unpleasant side effects [such as chemotherapy and radiotherapy (*3*)] is the acquisition of learned food aversions. Pediatric cancer patients form significant aversions to specific foods consumed before a drug treatment that induces nausea and vomiting (*4*). Thus appetite loss in these patients may be due not only to the direct effects of these treatments but to aversions learned as a consequence of the association of foods with these side effects.

Learned food aversions may be of more general significance if learned aversions develop in response to the association of a diet with aversive physiological effects of the tumor itself. Thus, tumor-induced appetite loss, like drug-induced appetite loss, may be based indirectly on learned aversions, with the unconditioned stimulus being some chronic unpleasant symptoms of tumor growth rather than the acute effects of a drug injection. In our studies, we examined the food intake and diet preference of rats bearing transplantable tumors and found that when a novel diet was introduced to anorexic, tumor-bearing animals, (i) the new food was strongly preferred to the food eaten during tumor growth and (ii) food consumption was immediately elevated. These results suggest that the development of learned aversions to the specific diet eaten during tumor growth contributes to tumor anorexia.

In our experiments, we subcutaneously implanted the flanks of male syngeneic Wistar-Furth (W/Fu) rats (M.A. Bioproducts) with 40- to 60-mg pieces of polyoma virus–induced sarcoma [PW-739 (*5*)]. Control animals received an incision and suture but no tumors were implanted. (The PW-739 tumor grows progressively and is lethal to animals within 8 to 12 weeks. In addition, we have found (*6*) that the growth of this tumor is associated with anorexia and cachexia; a marked decline of food intake and body weight typically begins approximately 5 to 6 weeks after the tumor is implanted.) In all three studies, animals were housed

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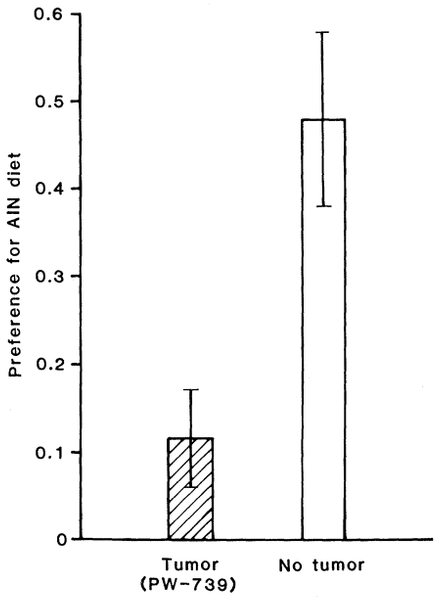

Fig. 1. Average preference [± standard error of the mean (S.E.M.)] for AIN meal in the 24-hour two-food choice test (AIN meal versus chocolate chow). Average total consumption: tumor, 18.3 g; control, 17.4 g.

in groups for 5 to 6 weeks after implant, with free access to Purina rat chow and water; they were then housed individually, and diet exposure began. During this time, food intake, body weight, and tumor size were measured daily.

In study 1, tumor-bearing (N = 8) and control (N = 8) rats were provided with a complete semisynthetic diet [American Institute of Nutrition (AIN) meal (*7*)] on a continuous basis for 10 days. The next day, preference for the AIN meal was evaluated by offering two diets [AIN and a novel diet (*8*)] simultaneously for 24 hours and measuring consumption of each. Preference scores for the AIN diet were calculated for each animal by dividing AIN diet consumption by total food consumption over the 24-hour test.

During the 10-day diet exposure period, intake of the AIN diet by tumor-bearing rats declined considerably below that of controls. Mean intakes on the last 2 days of this period were 9.9 g for tumor-bearing rats, compared with 15.7 g for controls [$t(14) = 2.54$; $P < .05$]. Mean AIN preference scores (Fig. 1) indicate that tumor-bearing animals appeared to have developed a pronounced aversion to the AIN diet, which had been available during recent tumor growth. They had a significantly lower preference for that diet than normal controls did [$t(14) = 3.36$; $P < .01$]. In addition, striking elevations of 24-hour food consumption were seen in tumor-bearing animals but not in controls when an alternate diet was available. Average food intake of tumor-bearing animals during the choice test was 85 percent higher than the average intake of the two previous days. Thus tumor-bearing animals, which had been consuming considerably less AIN diet than controls had, ate as much as controls did when a novel diet was introduced.

These initial results suggest that in rats anorexic because of the growth of PW-739 tumors, aversions develop to the specific diet eaten during recent tumor growth. Further, the observation that food consumption immediately increases when another diet is offered is compatible with the hypothesis that learned diet aversions contribute to the depression of food intake. It is possible, however, that the observed aversion to the AIN diet was not a learned response but a direct effect of the tumor on taste preferences. DeWys (*9*) has reported that tumor growth alters the taste responsiveness of cancer patients as well as of laboratory animals. Therefore, the differences we observed between tumor-bearing and control animals in their preference for the AIN diet could have been due to tumor-induced changes in taste responsiveness.

To distinguish this possibility from the hypothesis that these aversions were specific to the food consumed during tumor growth, additional groups of tumor-bearing and control animals received continuous access to one of two diets for 21 days (study 2). Half of the animals in each group (N = 7) received a modified AIN diet, the other half, a modified NIH-07 diet (*10*). On day 22, rats were offered both AIN and NIH-07 diets for 24 hours. Consumption of the diet associated with tumor growth and the novel diet was measured. Preference scores for the associated food were calculated for individual animals by dividing the consumption of the associated diet during the preference test by total food consumption during that period. This counterbalanced design has been used (*11*) to evaluate the specificity of learned aversions.

Tumor-bearing animals had lower preferences for the associated food than did controls (Fig. 2). Tumor growth had a significant effect on preference for the associated diet [$F(1, 24) = 11.66$, $P < .005$]; the interaction of tumor and diet was not significant [$F(1, 24) = 1.64$; $P > .05$]. These results are compatible with the hypothesis that tumor-bearing animals acquire a specific learned association of a particular food with some physiological effect of the tumor. Although animals with AIN paired with tumor growth showed mean preference of only 0.17 for the AIN diet, those exposed to NIH-07 showed a mean preference of 0.88 for AIN. Therefore, tumor growth alone is not a sufficient condition for the development of an aversion to the AIN diet (*12*).

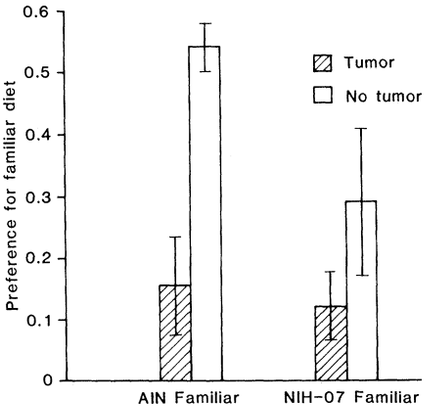

Fig. 2. Average preference (± S.E.M.) for the familiar or exposed diet in the 24-hour choice test (AIN meal versus NIH-07). Average total consumption: (AIN familiar) tumor, 12.0 g; control, 16.6 g. (NIH familiar) tumor, 20.1 g; control, 19.7 g.

We evaluated the generality of tumor-associated aversions in study 3 by exposing tumor-bearing rats to diets different from the ones used earlier. As in the previous studies, tumor-bearing and control animals received continuous access to one of two diets (N = 8)—ground Purina monkey chow or a soy meal diet (*13*). Exposure to these diets lasted 8 days; on day 9, rats were offered both diets simultaneously for 24 hours. Once again, tumor-bearing animals had lower preference for the associated food than did controls; the effect of tumor growth on diet preference was significant [$F(1, 28) = 7.07$; $P < .025$] (*14*). Thus, tumor growth diminished the preference for a wide range of complete diets. Additional research has indicated that these results do not reflect an enhanced preference for novel foods (rather than a specific aversion) in tumor-bearing animals (*15*).

We found that tumor-bearing animals (i) consumed significantly less food than controls at the end of the diet exposure period, (ii) showed significantly lower preferences for the diet consumed during tumor growth, and (iii) increased food intake when a new diet was available during preference tests so that their intake was no longer significantly lower than that of controls. Taken together, these findings suggest that the growth of an anorexigenic tumor produces learned aversions to the available diet and that introducing a new diet can produce an

immediate, although probably temporary, increase of food intake. It remains to be determined whether other anorexigenic tumors induce diet aversions, although it seems unlikely that this effect would be unique to the PW-739 tumor. Further studies should also evaluate the extent to which learned food aversions contribute to the overall anorexic effect of tumor growth.

Our results are reminiscent of the work of Rozin and co-workers (*16*) showing that the anorexia and weight loss of rats with vitamin deficiencies are associated with a learned aversion to the deficient diet. Rats deficient in thiamine, for example, demonstrate an aversion to their thiamine-deficient diet by spillage and a strong preference for any new diet offered them. Rozin concluded that the anorexia characteristic of many vitamin deficiencies reflects, at least in part, a learned aversion to the deficient diet since the anorexia symptoms disappear when a new diet is offered.

Food aversion learning enables rats and many other species to learn to select needed nutrients (*16*) and avoid toxins (*17*). This mechanism allows organisms to associate the delayed internal effects of toxins and imbalanced nutrients with the taste of consumed foods and to adjust their intake and preferences accordingly. However, this mechanism may be triggered under inappropriate circumstances, as in the case of the tumor-bearing animal which associates its tumor-induced discomfort and illness with its food. Food aversion learning seems to play a role in the anorexia produced by certain tumors. Since food aversion learning occurs in humans in a variety of circumstances (*4, 18*), these findings may be of clinical importance. An understanding of the factors controlling the acquisition of these aversions may enable assessment of the degree to which they contribute to cancer anorexia and may suggest methods of preventing them.

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References and Notes

1. J. C. B. Holland, J. Rowland, M. Plumb, *Cancer. Res.* **37**, 2425 (1977).
2. G. Costa, *ibid.*, p. 2327; P. Baillie, F. K. Millar, A. W. Plumb, *Am. J. Physiol.* **209**, 292 (1965); J. Gold, *Ann N.Y. Acad. Sci.* **230**, 103 (1974); A. Theologides, *ibid.* **230** (1974); S. D. Morrison, *Physiol. Behav.* **17**, 705 (1976).
3. S. S. Donaldson, *Cancer Res.* **37**, 2407 (1977); T. Ohnuma and J. F. Holland, *ibid.*, p. 2395; G. Costa and S. S. Donaldson, *N. Engl. J. Med.* **300**, 1471 (1979).
4. I. L. Bernstein, *Science* **200**, 1302 (1978); I. L. Bernstein, M. J. Wallace, I. D. Bernstein, W. A. Bleyer, R. L. Chard, J. R. Hartmann, in *Nutrition and Cancer*, J. van Eys, M. S. Seelig, B. L. Nichols, Eds. (Spectrum, New York, 1979), p. 159.
5. This polyoma virus-induced sarcoma was induced in syngeneic W/Fu rats by the intraperitoneal inoculation of approximately 2.5×10^6 polyoma virus particles into W/Fu rats within 24 hours of birth. Primary polyoma tumors arose in the kidneys of the injected animals within 6 weeks and were established by subcutaneous transplants.
6. I. L. Bernstein, H. H. Samson, R. A. Sigmundi, in preparation. Anorexic tumor-bearing animals were autopsied, and metastases were noted in the draining lymph nodes but not in the abdomen.
7. American Institute of Nutrition, *J. Nutr.* **107**, 1340 (1977). The AIN-recommended diet consists of casein (20 percent); cornstarch (15 percent); sucrose (50 percent); fiber (5 percent); corn oil (5 percent); mineral mix (3.5 percent); vitamin mix (1 percent); choline bitartrate (0.2 percent); and DL-methionine (0.3 percent).
8. Novel diet of comparable palatability to the AIN diet (in untreated animals) was composed of five parts ground Purina rat chow; five parts Carnation chocolate instant breakfast mix, and one part Crisco vegetable oil, by volume.
9. W. D. DeWys, *Ann. N. Y. Acad. Sci.* **230**, 427 (1974).
10. NIH-07 open-formula rat and mouse ration consists of dried skim milk (5 percent); fish meal (10 percent); soybean meal (12 percent); alfalfa meal (4 percent); corn gluten meal (3 percent); ground shelled corn (24.5 percent); ground wheat (23 percent); wheat middlings (10 percent); brewer's yeast (2 percent); dry molasses (1.5 percent); soybean oil (2.5 percent); and vitamin and mineral mixes (2.5 percent).
11. P. Rozin, *J. Comp. Physiol. Psychol.* **67**, 421 (1969); S. H. Revusky, in *Animal Memory*, W. K. Honig and R. H. R. James, Eds. (Academic Press, New York, 1971), p. 155.
12. Differences between the two control groups in their preference for associated diets are evident. Two factors contributed: (i) baseline palatability of the two diets is different, with the AIN diet being preferred to NIH-07 because it is high in sucrose. A counterbalanced diet design does not require diets of equal palatability (*11*); (ii) we have found, using a variety of complete diets, that exposure to a diet tends to produce a small reduction in consumption of that diet (relative to a novel diet) during a 24-hour choice test. Preferences for associated diets are lower than preferences for the same diets when they are novel. This applies to 24-hour choice tests, which are apparently long enough to overcome initial neophobic effects. These effects are small relative to the effects of tumor growth on diet preference, and, since tumor-bearing animals were compared with appropriate controls, these differences do not detract from our demonstration of specific aversions as a consequence of tumor growth. Differences in diet palatability in studies 2 and 3 also affected absolute consumption by tumor-bearing animals during the test, with those offered the familiar, aversive diet and an unpalatable choice showing somewhat less recovery than those offered a more palatable choice. In all studies, tumor-bearing animals' food intake was not reliably lower than that of controls.
13. A. I. Leschner, G. H. Collier, R. L. Squibb, *Physiol. Behav.* **6**, 1 (1971).
14. Mean food consumption by each group in the 2 days before the preference test was 14.3 g for tumor-bearing rats and 17.6 g for controls (in the soy meal group); 12.8 g for tumor-bearing rats and 17.7 g for controls (in the monkey chow group). During the preference test, mean food consumption was 18.7 g for tumor-bearing rats and 19.8 for controls (in the soy mean group) and 15.4 g for tumor-bearing rats and 18.2 g for controls (in the monkey chow group).
15. In a separate study, tumor-bearing animals had continuous access to Purina monkey chow during the latter stages of tumor growth. When offered a choice between a novel diet (NIH-07) and the familiar diet they had eaten before the tumor implant (Purina rat chow), they preferred the familiar diet (85 percent). Normal, naïve rats given this choice consumed more NIH-07 (26 percent of diet consumed was Purina rat chow).
16. P. Rozin, *J. Comp. Physiol. Psychol.* **64**, 237 (1967); in *Handbook of Physiology: Alimentary Canal*, C. F. Code, Ed. (Waverly, Baltimore, 1967); _____ and J. W. Kalat, *Psychol. Rev.* **78**, 459 (1971).
17. J. Garcia, W. G. Hankins, K. W. Rusiniak, *Science* **185**, 824 (1974).
18. J. L. Garb and A. J. Stunkard *Am. J. Psychiatry* **131**, 1204 (1974); F. Lemere and W. L. Voegtlin, *Q. J. Stud. Alcohol* **11**, 199 (1950); F. L. Boland, C. S. Mellor, S. Revusky, *Behav. Res. Ther.* **16**, 401 (1978).
19. We thank I. D. Bernstein for providing the tumors used in these studies and R. Bolles, S. Woods, M. Fanselow, and M. Webster for their critical reading of the manuscript. Supported by grant R01-CA26419-01, awarded by the National Cancer Institute.

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The Testicular Feminized Rat: A Naturally Occurring Model of Androgen Independent Brain Masculinization

Abstract. *Although genotypically male (XY), the testicular feminized rat develops as an anatomic female because of an inherited deficiency in intracellular androgen receptors that prevents androgen imprinting of sexual primordia. However, the ability of testicular feminized rats to exhibit male-like sexual behavior and little feminine sexual behavior suggests that the brain can be masculinized without androgens.*

It has been generally concluded that the inherent program of sexual differentiation in both sexes of mammals is female. If androgens are present during sexual development then both genetic males and females will be organized for masculine reproductive organs (*1*), hepatic enzymes (*2*), hypothalamic control of gonadotropin secretion (tonic) (*3*), and sexual behavior (*4*), whereas absence of either gonad during the critical developmental period allows for the expression of the inborn female programs (*1–4*). Recently, it has been suggested that androgens per se are not necessarily required for masculine organization of the brain, and that it is estrogen that organizes the brain as male. According to the "conversion hypothesis," androgen secreted by neonatal males is converted to estrogen, and it is this metabolite that is active intracellularly (*5*). The administration of estrogen to neonatal animals can defeminize the brain (*4, 6*), and estrogen antagonists can block the masculinizing effects of neonatal androgens (*7*).

A naturally occurring model for studies of hormonal controls of sexual differentiation is the testicular feminized (Tfm) animal. Testicular feminization is a hereditary defect found in humans (*8*), cattle (*9*), mice (*10*), and rats (*11*) in

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