possible drug-induced changes in the responses with 0.2-Hz stimulation before the LTP episode. There was a 10-minute period between the end of the second LTP episode and the conditioning train for the third (wash) episode. During this time, input-output and baseline responses were again obtained. A final series of input-output responses was taken at the end of the experiment.
15. J. V. Levy, *Eur. J. Pharmacol.* **2**, 250 (1968); K. R. Courtney, *J. Pharmacol. Exp. Ther.* **213**, 114 (1980).
16. B. J. Clark, in *Beta Adrenoceptor Blocking Agents*, P. R. Saxena and R. P. Forsyth, Eds. (North-Holland, Amsterdam, 1976), pp. 45–76.
17. R. D. Hawkins, T. W. Abrams, T. J. Carew, E. R. Kandel, *Science* **219**, 400 (1983); E. T. Walters and J. H. Byrne, *ibid.* **219**, 405 (1983).
18. E. T. Walters and J. H. Byrne, *Soc. Neurosci. Abstr.* **9**, 168 (1983).
19. C. A. Breen and H. L. Atwood, *Nature (London)* **303**, 716 (1983).
20. D. Johnston and T. H. Brown, *J. Neurophysiol.* **50**, 464 (1983); T. H. Brown and D. Johnston, *ibid.* **50**, 487 (1983).
21. We thank F. J. Lebeda, E. T. Walters, and J. H. Byrne for helpful comments on the manuscript. This work was supported by NIH grants NS11535 and NS15772 and a McKnight Foundation Neuroscience Development Award.

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Uncertainty of Histologic Classification of Experimental Tumors

Konstantinidis *et al.* (*1*) reported that a single systemic dose of a rapidly metabolized carcinogen promoted the development of malignant tumors at sites of chronic inflammation. In support of this conclusion, they stated that 14 of 47 carcinogen-treated rats developed malignant "soft tissue tumors" at a focus of buccal mucosal irritation, whereas other carcinogen-treated or control rats developed only "hyperplasia and severe inflammatory infiltration." Although the authors implied that some rats died as a result of malignancy [see reference 4 in (*1*)], no actual data were presented regarding the biologic behavior of "malignant tumors" as opposed to benign or hyperplastic lesions. Instead, cellular proliferations apparently were classified as malignant tumors because of their histologic features (legend to table 1).

The use of histologic criteria to assess the "malignancy" of tumefactions in laboratory rodents (*2*) is particularly convenient in large studies of experimental carcinogenesis because it permits animals to be killed as soon as their "tumors" are palpable. The lesions then are classified on the basis of cytologic or histologic features generally associated with malignancy, such as hypercellularity, hyperchromatism, increased mitotic activity, and apparent local invasion. However, the predictive value of these criteria can vary markedly depending on tumor type, organ, and species. As a result, the validity of individual cytologic or histologic indicators of malignancy must be established separately for each histologic variety of neoplasm under investigation (*3*). Spindle cell proliferations, which accounted for 10 of the authors' 14 cases, are among the most difficult to classify by histology. In man, certain spindle cell lesions with extreme cellular pleomorphism and hyperchromatism rarely, if ever, metastasize and are generally cured by simple excision (*4*). Other benign proliferations of fibroblasts characterized by hypercellularity, mitotic activity, and apparent invasion of adjacent connective tissue may not even represent true neoplasms (*5*). In contrast to human spindle cell tumors, which have been studied extensively, spindle cell proliferations in murine rodents are poorly understood (*6*). Although they are among the more common experimentally induced "tumors," they rarely occur spontaneously. Furthermore, the widespread practice of killing experimental animals soon after their lesions have developed has left unanswered many questions about their natural history.

It has been recognized for decades [see, for example, (*7*)] that histologic confirmation of metastatic growth represents the most convincing proof of a tumor's malignancy. Alternatively, certain neoplasms that rarely metastasize are regarded as malignant because they grow relentlessly and invade contiguous normal structures such as blood vessels, muscle, or bone. Tumor size is an unreliable criterion of malignancy since even benign lesions can occasionally achieve great bulk (*8*). Once the natural history and histologic features of a particular class of tumors is well understood, it would seem reasonable, under certain circumstances, to evaluate these lesions by histology alone. This is clearly indicated in the management of human neoplasms because the objective is to intervene before the disease's natural history has become clinically evident. It is less easily justified in work with experimental animals, however, and is particularly unsuitable during investigations of new mechanisms of carcinogenesis.

Konstantinidis *et al.* (*1*) provided no data about tumor metastasis, pattern of local invasion, tumor size, or associated morbidity and mortality. Although, in their report, the authors referred to the lesions listed in table 1 as "histologically malignant," in my opinion the findings illustrated in figure 1 are difficult to distinguish from inflammation accompanied by fibroblast proliferation. Furthermore, all of the cytologic features mentioned in the figure legend may be exhibited by benign neoplasms or hyperplastic processes. The reference to the "dropping-off phenomenon" (legend to figure 1D) and the fact that the authors classified separately the nine "malignant soft tissue tumors" and the single "fibrosarcoma" suggest that they considered the former to be derived from epithelial cells rather than fibroblasts. Making this distinction is particularly difficult (*4*) and ideally should be confirmed by electron microscopy (*9*). Finally, the method used to determine the interval until tumor appearance should be clearly explained, since most rats presumably had preexisting lesions related to hyperplasia and inflammation.

The observations of Konstantinidis *et al.* may have important implications and should be supported by additional information about the actual biologic behavior of the induced lesions. Without such data, the nature of these lesions, and therefore the conclusions of the study, remain unsettled.

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References and Notes

1. A. Konstantinidis, J. B. Smulow, C. Sonnenchein, *Science* **216**, 1235 (1982).
2. R. L. Carter and F. J. C. Roe, *Br. J. Cancer* **23**, 401 (1969); R. L. Carter, *ibid.*, p. 408.
3. In epithelial tumors of the canine perianal gland [H. A. Smith, T. C. Jones, R. D. Hunt, *Veterinary Pathology* (Lea & Febiger, Philadelphia, ed. 4, 1972), p. 233], for example, the only reliable criterion of malignancy is individual cell invasion of adjacent connective tissue. Lesions that appear histologically malignant in other respects but lack invasion pursue a benign course. The canine cutaneous histocytoma (*ibid.*, p. 238) also satisfies many cytologic and histologic criteria generally associated with malignancy; it is hypercellular, hyperchromatic, and has numerous mitoses. It also invades subcutaneous fat. However, these lesions spontaneously regress or are cured by local resection.
4. Some authors regard all of these lesions as benign [A. W. Hudson and R. K. Winkelmann, *Cancer* **29**, 413 (1972); D. F. Fretzin and E. B. Helwig, *ibid.* **31**, 1541 (1973)]; others believe that some of them may represent low grade malignancies (H. L. Evans and J. L. Smith, *ibid.* **45**, 2687 (1980)].
5. A. P. Stout and R. Lattes, *Tumors of the Soft Tissues* (Armed Forces Institute of Pathology, Bethesda, Md., 1967), p. 24; F. M. Enzinger and S. W. Weiss, *Soft Tissue Tumors* (Mosby, St. Louis, 1983), p. 15.
6. V. S. Turusov, Ed., *Pathology of Tumours in Laboratory Animals: Tumours of the Rat* (International Agency for Research on Cancer, Lyon, 1973), vol. 1, part 1, p. 151.
7. R. A. Willis, *Pathology of Tumors* (Butterworth, London, ed. 4, 1948), p. 67.
8. It is important to recognize that any large neoplasm near a vital structure can cause morbidity or mortality. For example, a large, noninvasive, histologically benign tumor of the oral cavity or esophagus may result in death by inanition. Thus, lethality is not unequivocal proof of malignancy. Alternatively, certain unusual neoplasms with innocuous cytologic features have a relatively benign clinical course even though they spread by implantation [C. G. Julian and J. D. Woodruff, *Obstet. Gynecol.* **40**, 860 (1972)], vascular invasion, or even metastasis [H. J. Norris and T. Parmley, *Cancer* **36**, 2164 (1975)]. These lesions appear to occupy a border zone between clearly malignant and strictly benign neoplasms.

9. B. Lichtiger, B. Mackay, C. F. Tessmer, *Cancer* **26**, 1311 (1970); C. Leifer, A. S. Miller, P. B. Putong, B. H. Min, *ibid.* **34**, 597 (1974); H. Battifora, *ibid.* **37**, 2275 (1976).
10. I thank D. A. Antonioli, R. B. Cohen, J. L. Connolly, H. F. Dvorak, M. L. Goodman, R. D. Hunt, T. C. Jones, T. H. Kwan, and B. Z. Pilch for helpful discussions.

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Galli has provided a scholastic assessment of what constitutes malignancy in the eyes of clinical pathologists. The point of our report (*1*), however, was to provide empirical evidence for a significantly different interpretation of currently accepted mechanisms of carcinogenesis (*2*). For example, rats in groups 1 and 2 given a single intraperitoneal *N*-nitroso-*N*-methylurea (NMU) injection developed tumors at the site where a stainless steel wire irritated the buccal mucosa; no such tumors developed on the contralateral side of the same animals where no wire was placed. Also, rats without the irritating wire but given the same intraperitoneal dose of NMU, had no tumors in the buccal mucosa (group 3). Additionally, no tumors developed in rats when the wire was placed in the mouth, but no NMU was injected (group 4). The two control groups of rats were observed for periods of time comparable to the times for the test groups. From our perspective, the significantly increased rate of tumor formation in our test groups in comparison with control groups was sufficient to suggest that the carcinogen affected an event controlling cell proliferation and generated in a centrally located organ (the liver?); this, plus undefined local interactions of NMU on epithelial and fibroblast stem cells, together with the iterative nonspecific proliferative effect of the irritation by the wire, resulted in tumor formation. This was the extent of our claims resulting from the data collected. We realize that the diagnosis of a malignant lesion in a clinical setting has immediate prognostic and therapeutic implications. Our understanding of the "state of the art" in this context is that honest disagreements are not infrequent in judging whether histologic features represent actual or potential threats leading to the premature death of the host. Our report, however, was not intended to address clinical considerations.

Galli's comments detail more than was written or implied in our report (*1*); our reference 4 neither stated nor implied that rats died as a result of malignancies. We agree that the predictive value of histologic features of suspected malignant tumors varies with tumor types, organs, and species. We also share his concern regarding the need for more detailed studies to document the chronological events occurring prior to tumor formation, and we did this in a subsequent publication (*3*). Finally, Galli kindly and correctly states that our observations "may have important implications," and we share his recommendation regarding a more extensive follow-up on this research approach.

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References and Notes

1. A. Konstantinidis, J. B. Smulow, C. Sonnenschein, *Science* **216**, 1235 (1982).
2. C. Sonnenschein and A. M. Soto, in *Estrogens in the Environment*, J. A. McLachlan, Ed. (Elsevier/North Holland, New York, 1980), p. 169.
3. J. B. Smulow, A. Konstantinidis, C. Sonnenschein, *Carcinogenesis* **4**, 1083 (1983).
4. We thank R. DeLellis for helpful discussions.

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Impact Event at the Cretaceous-Tertiary Boundary: A Possible Site

The discovery of shock-metamorphosed quartz grains in the Cretaceous-Tertiary (K-T) boundary clay in eastern Montana (*1*) provides evidence for an extraterrestrial impact at that time. The site of the impact, however, remains a challenging question.

The site may be on the North American continent. The presence of quartz and sanidine in the target rock (*1*) indicates continental rather than oceanic target rock, and the unusually large size (for fallout material) of the mineral fragments (50 to 100 μm) implies that they were deposited relatively close to the impact site. Grains this large would have settling velocities in air of about 100 cm/sec (*3*) and would settle from stratospheric heights (30 to 50 km) in 18 to 36 hours. If the impact cloud spread at velocities observed for volcanic eruptions (about 100 km/hour) (*4*), then the grains would settle out within 3600 km of the impact site, again implying an impact site on the North American continent. Previously proposed sites (*5*) are all much farther from the eastern Montana collection site than 3600 km; for example, the two structures in the Soviet Union are about 15,000 km away.

At least two candidate impact structures do exist in North America: the Sierra Madera structure, Texas (*6*), and the Manson structure, Iowa (*7, 8*). Both structures have definite shock-metamorphic characteristics, and both are more than 10 km in diameter. Both are of less than Lower Cretaceous age, although neither structure has been accurately dated.

Of the two, Manson seems the stronger candidate. It is larger (minimum diameter, 32 km), closer to the collecting site (about 1150 km), and emplaced in granitic crustal rocks. Sierra Madera is smaller (16 km), farther from the collecting site (about 1850 km), and emplaced in sediments (chiefly limestones and shales) that contain little quartz. Manson is covered by about 30 m of glacial drift (*7*) and could be much larger than its current estimated diameter.

The volcanic cloud analogy may be inappropriate if the impact-produced dust was more widely distributed along ballistic trajectories (*9*) or by global atmospheric turbulence created by the impact event. Preliminary data on grain sizes of shock-metamorphosed quartz from K-T boundary sediments elsewhere (*10*) suggest that such mechanisms may indeed have operated. Such studies may also provide important data about atmospheric conditions immediately after the impact.

The Manson structure in particular should be studied in more detail to determine its true extent, its exact age, and its possible connection to the K-T impact event.

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References and Notes

1. B. F. Bohor, E. E. Foord, P. J. Modreski, D. M. Triplehorn, *Science* **224**, 867 (1984).
2. L. T. Silver and P. H. Schultz, Eds., *Geol. Soc. Am. Spec. Pap. 190* (1982).
3. G. P. L. Walker, L. Wilson, E. L. G. Bowell, *Geophys. J. R. Astron. Soc.* **22**, 377 (1971); L. Wilson, *ibid.* **30**, 381 (1972); D. M. Harris and W. I. Rose, Jr., *J. Geophys. Res.* **88**, 10,969 (1983); D. M. Harris, personal communication.
4. A. M. Sarna-Wojcicki, S. Shipley, R. B. Waitt, Jr., D. Dzurisin, S. H. Wood, *U.S. Geol. Surv. Prof. Pap. 1250* (1981), pp. 577–600; W. I. Rose, Jr., and M. F. Hoffman, *NASA Spec. Publ. SP-458* (1982), pp. 1–14; S. N. Carey and H. Sigurdsson, *J. Geophys. Res.* **87**, 7061 (1982); G. A. Macdonald, *Volcanoes* (Prentice-Hall, Englewood Cliffs, N.J., 1972), pp. 136–140; G. P. L. Walker, *J. Volcanol. Geotherm. Res.* **11**, 81 (1981).
5. W. Alvarez, L. W. Alvarez, F. Asaro, H. V. Michel, in (*2*), pp. 305–315; R. A. F. Grieve, *ibid.*, pp. 25–38.
6. H. G. Wilshire, T. W. Offield, K. A. Howard, D. Cummings, *U.S. Geol. Surv. Prof. Pap. 599-H* (1972).
7. R. A. Hoppin and J. E. Dryden, *J. Geol.* **66**, 694 (1956).
8. N. M. Short and T. E. Bunch, in *Shock Metamorphism of Natural Materials*, B. M. French and N. M. Short, Eds. (Mono Book, Baltimore, 1968), pp. 255–266; T. E. Bunch, *ibid.*, pp. 413–432.
9. O. B. Toon *et al.*, in (*2*), pp. 187–200; E. M. Jones and J. W. Kordis, *ibid.*, pp. 175–186.
10. B. Bohor, E. Foord, P. Modreski, D. Triplehorn, *Lunar Planet. Sci.* **15**, 74 (1984).

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Uncertainty of histologic classification of experimental tumors

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