involve opioid modulation of sympathetic outflow (38), also thought to regulate immune function (39).

Although knowledge of the precise involvement of the immune system in surveillance against tumors is limited, there is evidence to suggest that NK cells play a pivotal role (6). In this regard, we found that the same footshock stress regimen which causes a naltrexone-sensitive suppression of NK activity also enhances development of an immunogenic, experimental mammary ascites tumor (MAT 13762B) in F344 rats (40). This effect also was prevented by naltrexone, and the nonopioid stress did not significantly affect tumor growth. Despite the apparent correlation between the effects of opioid stress on the immune system and on tumor growth, it is premature to conclude that NK suppression mediates the tumor potentiating effect of stress. More generally, our findings support the view that the nervous system, by significantly modulating immune function, exercises some measure of control over the inception and development of certain disease processes. These results also reinforce continuing efforts to dissect the underlying mechanisms of stress in order to account for some of the variance prevalent in studies of this kind.

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- nisms of Pain, L. Kruger and J. C. Liebeskind, Eds. (Raven, New York, in press), vol. 6. A preliminary report of some of these data has been made [Y. Shavit, J. W. Lewis, G. W. Terman, R. P. Gale, J. C. Liebeskind, *Proc. West. Pharmacol. Soc.* 26, 53 (1983)]. The opioid and nonopioid bases of these two stress analgesias was determined in a separate study in which F344 female rats were injected with either naltrexone (10 me/kg subcutaneous 15
- bit of the matter source of the matching when the matter source of the matter source of the matching subcomposition N = 12. Twenty minutes later, half the animals in each group were subjected to the intermittent and half to the continuous footshock paradigm. All rats were then tested for analgesia using the tail-flick test. Stress analgesia was significantly attenuated by naltrexone in rats exposed to the intermittent, but not the continuous, footshock. 17. RPMI 1640 media was supplemented with 0.1
- percent gentamicin, 1 percent 1-glutamine, and 10 percent heat-inactivated fetal calf serum.
- To percent heat-inactivated fetal call serum. YAC-1 cells were propagated in tissue culture (in complete RPMI 1640 further supplemented with 1 percent sodium pyruvate and 1 percent nonessential amino acids) at  $37^{\circ}$ C, in a humidi-fied atmosphere containing 5 percent CO<sub>2</sub>. Cells were labeled with chromium-51 by incubation for 2 hours at  $37^{\circ}$ C. Labeled cells were washed and resurgended in complete RDMI 1640 at a 18.
- and resuspended in complete RPMI 1640 at a concentration of 10<sup>5</sup> cells per milliliter.
  Percent specific cytotoxicity was calculated using the formula (w s)/(m s) · 100, where w is counts per minute in the test well, m is the maximum release counts per minute, and s is maximum release. spontaneous release counts per minute. Test well counts per minute were determined in 100  $\mu l$  supernatant from wells in which effector and target cells were cocultured. Portions of 100  $\mu l$ of supernatant from wells in which only YAC-1 cells were incubated served to determine both spontaneous and maximum release counts per minute. In a typical assay, percent specific cytotoxicity for untreated control animals was
- Group and the second se 20.
- We have recently found that tolerance develops to morphine's immunosuppressive action when rats are given the drug once daily at a dose of 30 mg/kg for 14 days. This finding suggests that morphine's effect is via opioid receptors. That

such high doses (> 10 mg/kg) are required to alter NK activity suggests either mediation by receptors with low affinity for this drug or the Perceptors with low almity for this grug of the necessity of receptor occupation for prolonged periods of time. Y. Shavit, J. W. Lewis, G. W. Terman, R. P. Gale, J. C. Liebeskind, *Soc. Neurosci. Abstr.* 8, 11 (1982)

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# Analysis of the Cretaceous-Tertiary Boundary Clay: **Methodology** Questioned

Rampino and Reynolds' study (1) of the clay mineralogy of Cretaceous-Tertiary (K/T) boundary clays from four localities concludes that these boundaryclay samples are neither mineralogically exotic nor distinct from clays above and

below the boundary. Furthermore, Rampino and Reynolds could not detect the significant ejecta component in these boundary clays that is predicted by the asteroid-impact scenario, and they propose that volcanic material be considered as an explanation of the geochemical anomalies described at the K/T boundary. I wish to point out that their choice of analytical methods precludes the attainment of their stated objectives and that their data do not support the discarding of the asteroid-impact hypothesis.

First, their study encompassed only the  $< 2-\mu m$  fraction of the boundary clays. With the elimination of all components larger than 2 µm, surely most of the mineralogically exotic materials expected from an asteroid impact will have been missed. If one wishes to distinguish this material from ordinary volcanic ejecta or land-derived detrital components, then one must look at the size fraction in which they would most probably occur (> 2  $\mu$ m). Studies of altered volcanic ashes (tonsteins) in coal beds have shown that the nonclay mineral components that characterize these rocks as volcanic in origin are much larger than 2  $\mu$ m (2). To ignore this larger-sized component almost eliminates any possibility for determining whether the samples are volcanic or not.

Can the clay fraction  $< 2 \mu m$  be useful at all in determinations of origin? By these investigators' own admission (1), the glassy phase of fine ejecta from an asteroid impact might be altered to a clay mineral such as smectite, which might be difficult to distinguish from the volcanogenic smectite present in the marine boundary sections. Glasses of similar compositions, regardless of origin, would be expected to alter to similar clay minerals on the ocean floor. Clay minerals in the limestone and marl beds enclosing the boundary clays also would be expected to be derived mostly from ubiquitous volcanic glass settling from the water column along with the calcareous component. The Late Cretaceous, after all, was a time of intense volcanism, as pointed out by Rampino and Reynolds (1). Thus, would one expect to find large differences in clay mineralogy between the boundary clays and the surrounding rocks?

Second, the method of sedimenting the clay fraction (< 2  $\mu$ m) onto glass slides for x-ray diffraction analysis was a poor choice for this study. Gibbs (3) and many other workers since have shown that sedimentation onto glass slides allows size fractionation to occur, masking coarser components with finer-sized clay minerals. Thus, diffraction from the finest clay-sized material of the  $< 2-\mu m$ size fraction will be preferentially enhanced on the x-ray pattern. If any exotic material from the impact event were present in the < 2-µm fraction, the likelihood of its being revealed on the x-ray diffraction pattern with this type of mount is remote.

In summary, because of the choice of analytical methods, the conclusions drawn from this study are based on incomplete data and do not represent a clear assessment of the available mineralogical information from these samples. We still do not know whether these marine K/T boundary clays represent detrital, volcanic, or extraterrestrial events. Consequently, the asteroid-impact hypothesis remains alive and well and living in Berkeley.

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Bohor seems to have missed the point of our investigation. We did not undertake this study to prove or disprove a volcanic origin for the clay layer but to perform what seemed to be a clear-cut test of one of the predictions of the asteroid-impact model, namely, that the fine-fraction mineralogy of the layer should give some indication of an exotic origin.

We concentrated on the  $< 2-\mu m$  fraction specifically because this is the material with significant stratospheric residence times that would be widely dispersed to form the dust cloud of the impact model (1). We avoided the > 2µm fraction precisely because it could (and in fact does) contain local contaminants (2). Investigators who have examined the fraction of the boundary layers coarser than 1 µm report no common carrier phase for the supposedly extraterrestrial elements (3). Our main find-

## **Receptor Binding Studies**

In their recent discussions of receptor binding and Scatchard plots, neither Klotz (1) nor Munson and Rodbard (2) addressed problems in the estimation of free ligand concentrations. In general, the tissue concentrations of cellular receptors are very low and their binding activities are unstable. Consequently, reings were that the boundary layers were different mineralogically at each locality studied and that the clay layers were similar to clays stratigraphically above and below them, in contrast to the predictions of an impact origin (4).

The glass slide method does indeed enhance the diffraction contribution from the finest grain sizes and diminishes the intensities of diffraction from the coarser material. However, it is much too strong to say that this method makes detection of the coarser material in the  $< 2-\mu m$  fraction remote. In the usual case, intensities may be reduced by 20 to 30 percent of the amount present for minerals concentrated near the bottom of the slide. We claim approximately a 5 percent detection limit; the differences between 5 and 6 or 7 percent are insignificant.

We agree with Bohor that it is difficult to differentiate between smectite produced by volcanic glass and smectite formed from glassy impact debris. Our position here was based largely on philosophical grounds. In the context of Ockham's razor, a line of reasoning seems unnecessarily contrived if it requires that one of the altered glassy horizons in Upper Cretaceous rocks has an origin that is qualitatively different from the many that are clearly of volcanic derivation.

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ceptor interactions with hormones and other effector molecules have been studied almost exclusively with rapid, nonequilibrium methods that separate and quantify only receptor-bound radioligands. Although the free ligand concentrations that existed at equilibrium prior to the separation step are required for