

## Corticosterone Concentrations in the Mouse

The report of Monjan and Collector (1) emphasizes the modulating effects of stress on the immune response in mice. This important and timely contribution strengthens a growing awareness that either intentional or uncontrolled anxiety-stress in various forms can have critical effects on immunological and pathological reactions in mice and other experimental animals. There are some technical matters in their report, however, that require clarification.

The authors' use of the terms "cortisol" and "cortisone" when referring to circulating plasma glucocorticoids in the mouse may have been inadvertent, but is, of course, scientifically incorrect. Unlike the case in man, dogs, rabbits, and certain other mammals that produce both cortisol and corticosterone, recent experiments have shown that in the mouse corticosterone is the only glucocorticoid found in the plasma (2-4). Using a microfluorescence assay and a column chromatographic separation procedure, we have demonstrated that cortisol is not present in plasma obtained from either normal quiescent mice or from mice that have been stressed in various ways. Even in pregnant mice where plasma concentrations of corticosterone were increased to 3600 ng/ml, no cortisol was present (5). It is also unlikely that "cortisone" could be present to act on lymphocytes since cortisone is not secreted by the adrenal gland and is normally found only in the liver of cortisol-producing mammals as a short-lived metabolite of cortisol (6).

The radioimmune assay (RIA) kit employed by Monjan and Collector was designed for the measurement of cortisol in patients and other mammals where the predominant circulating glucocorticoid is cortisol. That this RIA cortisol procedure cross-reacts with corticosterone to an extensive degree is evident from their results. However, since the extent of the cross-reaction is unknown, it is difficult to deduce the actual plasma corticosterone concentrations based on the "cortisol" values which were reported.

Additional items of information needed for a better evaluation and appreciation of their important results are: (i) the time of day at which blood samples were obtained from their stressed mice and (ii) the specific procedures employed in handling the mice and in obtaining blood samples. The fluctuating concentration of plasma corticosterone in the mouse follows a circadian rhythm, ranging between 5 and 35 ng/ml in the morning and early afternoon but increasing to over 200 ng/ml between 7 and 10

p.m. (2, 3, 7). Monjan and Collector state that their mice were exposed to a noise-stress program during a 1- or 3-hour period around midnight, but the time and manner of the subsequent blood collections were not given. The procedures used are relevant in that mice respond with great rapidity to the stresses of conventional handling techniques. Thus, if more than 4 minutes elapse from the time that the quiescent mice are removed from the shelf until the blood is collected, stress-induced increases in plasma corticosterone, which increase with elapsed time, will be obtained (2, 8). Such increased hormone concentrations may be as much as 20 times higher than the authentic concentrations in quiescent mice (2, 3).

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### References and Notes

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We are pleased to clarify some of the points raised by Spackman and Riley in reference to our report. We accede to their data indicating that corticosterone is the primary, if not only, glucocorticoid circulating in mouse plasma. The anti-serum in the radioimmunoassay kit (New England Nuclear Cortisol <sup>3</sup>H Reagent Pak) that we used is not monospecific for cortisol. Thus, while we attempted to reduce cross-reactivity with corticosterone by column elution through Sephadex LH-20, we must assume, on the basis of the data given by Spackman and Riley, that an unknown degree of cross-reaction did occur. Therefore, our figure 2A reflects relative circulating adrenal corticosteroids.

In response to the other procedural questions that were raised, we routinely killed the mice between 9 and 10 a.m. Animals were removed from their home cages, decapitated, and trunk blood was collected within 1 minute. We have observed that more traditional and traumatic procedures such as retro-orbital bleeding produce massive perturbations of the hypophyseal-adrenal axis which can have residual effects on splenic lymphocytes even after 3 days in tissue culture.

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## Migration of Plutonium in Rock: Incorrect Dispersion Formula

Fried *et al.* have reported some very interesting laboratory work on the retention of plutonium and americium by rock (1). I particularly welcome their attempt to experimentally model a fissure, in view of the possibility that, in the event of a release of radioactive waste underground, the bulk of the waste isotopes might travel through fissures.

However, the sweeping conclusion that Fried *et al.* reach in their last sentence, namely, that "on the basis of our conservative estimates, the boundary for . . . 'safe' Pu concentrations in a real [ra-

dioactive waste depository] site will be much less than 50 km," is not substantiated by the data and analysis presented. The data are quite limited, and the analysis is based on an incorrect dispersion formula.

Fried *et al.* assume a Gaussian distribution with

$$\sigma = (2\bar{d})^{1/2} \quad (1)$$

for the longitudinal dispersion during the migration through an aquifer of an initially localized plutonium deposit. In Eq. 1,  $\sigma$  is the full width of the dispersion at