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 coritsol 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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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 antiserum in the radioimmunoassay kit (New England Nuclear Cortisol ^{3}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 [radioactive 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

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

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

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half maximum and $\bar{d}$ is the mean distance of its migration. Dimensional reasoning indicates that this relationship is incorrect.

Looking up the reference cited by Fried *et al.* for the above dispersion formula (*2*), I find that they have fallen victim to an erratum. In (*2*) the correct dispersion formula (*3*)

$$\sigma_L^2 = \frac{1}{3}(\lambda + 0.173)\bar{d}\ell \qquad (2)$$

is reproduced with the grain size, ℓ, omitted. In Eq. 2. σ_L is the standard deviation of the longitudinal dispersion (*4*) and λ is related to the ratio of the mean migration distance and the grain size through the formula

$$\frac{3\bar{d}}{\ell} = e^{2\lambda}(\lambda - 2.077) \qquad (3)$$

For a macroscopically homogeneous medium, a conservatively large grain size would be $\ell = 1$ cm. For $\bar{d} = 50$ km, this gives $\lambda = 7.43$ and σ, which is given (in units of kilometers) by

$$\sigma = 0.84 \times 10^{-2}(2\bar{d})^{1/2}$$

becomes more than 100 times smaller than implicitly assumed by Fried *et al.* As a result, the plutonium concentrations would be increased by a factor of 100.

Of course, in the real world the medium would not be homogeneous over a distance of 50 km, and fractures, layering, and other factors would lead to an effective grain size which might be of the order of tens of meters. Under these more realistic circumstances, the consequences of the error made by Fried *et al.* would be less severe.

However, the sweeping conclusions drawn by Fried *et al.* concerning the hazards associated with the release of transuranic elements from geological depositories are based on rather limited laboratory-scale experiments with the transuranic elements in ionic form and with rock types not including rock salt, granite, and shale, which are the rock types currently being considered as geologic media for the disposal of radioactive wastes. Conclusions about hazards will require a much broader base of data and analysis. In particular, the problem of the mobility of transuranic elements in organic complexes that are soluble in water will have to be confronted since the migration of these elements in those chemical forms would be essentially at groundwater velocities rather than at the slower rates, 10^3 to 10^4 times slower, that were measured by Fried *et al.* for ionic migration.

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2. J. Bear, in *Flow Through Porous Media*, R. J. M. De Wiest, Ed. (Academic Press, New York, 1969), chap. 4, p. 140. Bear's symbol L for the mean migration distance has been changed to $\bar{d}$.
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4. The relationship between σ and σ_L for a Gaussian dispersion function is $\sigma = 2.35\ \sigma_L$.

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We thank Krugmann for pointing out the error in the reference to J. Bear in our report (*1*). It should be noted, however, that this reference refers to hydrodynamic dispersion without absorption. It has recently been brought to our attention that (*2*) the relationship between the effective longitudinal dispersion parameter (K_L) and the concentration dispersion (σ) can be given as a function of migration time (t), in our case 400,000 years per 50 km. On the basis of this relationship, it is possible to account for the fact that the migrating species moves at a much slower rate than the solvent.

In (*2*) Fried and Combarnous state that

$$\sigma = (2K_L t)^{1/2} \qquad (1)$$

and that

$$K_L = \left(\frac{K_L}{D}\right)_0 + \beta u d \qquad (2)$$

where D is the diffusion coefficient, $\beta = 1.8 \pm 0.4$, u is the solvent velocity = 0.1 km/year, and d is the grain size = 1 cm or 10^{-5} km. The first term can be neglected (*2*, p. 227), and so we have

$$K_L = 1.8 \times 0.1 \times 10^{-5} = 1.8 \times 10^{-6}\ \text{km}^2/\text{year}$$

and Eq. 1 becomes

$$\sigma = [2(1.8 \times 10^{-6})(4 \times 10^5)]^{1/2} = 1.20\ \text{km}$$

If we use Krugmann's estimate of the real grain size as 10 m, then

$$\sigma \approx 38\ \text{km}$$

The important parameter is the amount of radioactivity at any time at the site boundary. The dispersion affects this radioactivity in two ways: a large dispersion tends to cause greater dilution, and a large dispersion will cause radioactive material to be present earlier than the major peak. The interplay between these effects is most important in determining the geological retention of radioactivity by the strata.

Krugmann's caveat that our laboratory results cannot be extrapolated to geological media is quite valid. In fact, we stated in our conclusion (*1*) that "rock strata from actual proposed sites must be tested for any realistic evaluation."

It should be noted in answer to Krugmann's last paragraph that organic complexing agents are generally absent in deep groundwater. It is therefore unlikely under these conditions that organic complexes could be formed with actinides which might move with groundwater velocities. In addition, such organic complexing agents would be much more likely to form complexes with the vastly greater concentrations of ions such as calcium already in solution.

Finally, we have tried to be very conservative in our estimates. We have ignored the finite size of the repository which will give us an initial dispersion of at least hundreds of meters. We have also postulated that all of the radioactivity is injected as an instantaneous single pulse. This is not credible in a real repository since the waste will be stored in insoluble form in a large array of canisters (*3*).

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29 August 1977; revised 23 January 1978