pletely than had been planned in the past, at additional but apparently tolerable expense. It is both technically and economically feasible to recycle the long-lived actinides (plus iodine, if desired) through the nuclear reactor; thus, the waste requiring disposal becomes benign after 700 years. The separation task is not trivial, especially for materials contaminated with traces of actinides, but we believe that very substantial advances can be made even there.

Under these circumstances where there is no need to consider geologic times, ocean disposal appears worthy of additional consideration. However, several problems remain, some of which are also discussed by Nielsen. (i) The technology is not yet good enough. In particular, devitrification and consequent dispersion into the environment of the glassified wastes are possible; making the waste pellets small does not reduce the damage caused by short-range particles. (ii) Regions with appropriately small ocean currents and other desirable qualities are hard to find. (iii) The oceans are increasingly subject to national and international control, so decisions about their use are no longer unilateral. (iv) Opposition may arise because people just don't like the idea. (v) Some mistakes would lead to very serious consequences.

These problems are not necessarily unsolvable; if ocean disposal becomes possible, we suspect that some kind of burial will be better than simple dumping. Note also that better separation of the various waste categories makes most other disposal options (such as salt mines or granitic structures) more attractive, too, and the whole assessment alters.

Nielsen makes an important point about the nuclear waste problem in Europe. The matrices of sites for nuclear power plants and fuel reprocessing plants and of acceptable disposal sites just do not match up within the national boundaries of Europe, and transnational solutions are required. Some aspects of this have been discussed elsewhere (*1*).

The U.S. Atomic Energy Commission (and other agencies elsewhere) could help to clarify views about disposal options by saying more about the possibility of better nuclear waste separation.

Nielsen agrees with us on most of the points made here.

DAVID J. ROSE
Nuclear Engineering Department,
Massachusetts Institute of Technology,
Cambridge 02139

ARTHUR S. KUBO
U.S. Military Academy,
West Point, New York 10996

References

1. D. J. Rose and G. Tenaglia, *Ambio* **2**, 233 (1973).

6 August 1974

Wilson's Disease and Copper-Binding Proteins

The application of Scatchard's method (*1*) to estimate the number and association constants of a protein's binding sites for a small molecule is valid only if the binding reaction is reversible and if the measurements are made at equilibrium. Since these criteria do not seem to be satisfied by the binding of copper to the hepatic protein described by Evans *et al.* (*2*), doubt must be cast on their conclusions.

The copper-binding protein, metallothionein, studied by these authors is very similar to the hepatic copper-binding protein, L-6-D, isolated and investigated by Morell *et al.* (*3*). Both proteins were extracted from homogenates of human liver by 0.025*M* phosphate buffer and, although one was purified by ethanol-chloroform precipitation and the other by chromatography, both were then soluble when dialyzed against distilled water or 0.005*M* phosphate buffer and both were lyophilized from the dialyzed solution.

The copper of L-6-D, as isolated from the liver, is bound so tightly to sulfhydryl groups that it cannot be freed by dialysis or ion exchange resins. The copper of the copperthionein studied by Evans *et al.* is also very tightly bound. In preparing apoprotein for their experiments, they removed the copper from the purified metallothionein in solution by chromatography at *p*H 2. Then, to obtain their experimental results, they dialyzed the apoprotein against several different concentrations of copper sulfate at *p*H 7.4. If equilibrium exists between copper and copperthionein at *p*H 7.4, then it should have been possible—and would have been preferable—to remove copper from the protein by dialysis at this *p*H. Since the copper of another copper-binding protein, ceruloplasmin, is irreversibly bound to the protein at *p*H 7.4 but is readily freed by dialysis below *p*H 3 (*4*), it seems essential to demonstrate directly the reversibility of the copper-protein bond of copperthionein under the conditions of the experiments.

However, whether or not copperthionein and L-6-D are similar, it is almost certain that copper is bound as cuprous ions to the sulfhydryl groups that characterize both proteins (*5*). So if equilibrium did exist between the free cupric ions of copper sulfate and the protein-bound cuprous ions, a complex, reversible oxidation-reduction reaction involving reversible binding of cuprous ions would have to be postulated. Without experimental evidence for the existence of such a system (*5*), equilibrium can hardly be assumed, and a Scatchard plot to quantitate and characterize the binding sites cannot be used.

If equilibrium was attained, the binding constants reported are each eight times too large. The intercept on the abscissa is *nk*—and not simply *k*—where *n* is the number of identical binding sites on the protein and *k* is the intrinsic association constant of each for copper.

I. HERBERT SCHEINBERG
Division of Genetic Medicine,
Department of Medicine,
Albert Einstein College of Medicine,
Bronx, New York 10461

References

G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
2. G. W. Evans, R. S. Dubois, K. M. Hambidge, *Science* **181**, 1175 (1973).
3. A. G. Morell, J. R. Shapiro, I. H. Scheinberg, in *Wilson's Disease, Some Current Concepts*, J. M. Walshe and J. N. Cumings, Eds. (Blackwell Scientific, Oxford, England, 1961), pp. 36–42.
4. C. G. Holmberg and C.-B. Laurell, *Acta Chem. Scand.* **2**, 550 (1948).
5. P. Hemmerich, in *The Biochemistry of Copper*, J. Peisach, P. Aisen, W. E. Blumberg, Eds. (Academic Press, New York, 1966), pp. 15–32; I. M. Kolthoff and W. Stricks, *J. Am. Chem. Soc.* **73**, 1728 (1951); I. M. Klotz, G. H. Czerlinski, H. A. Fiess, *ibid.* **80**, 2920 (1958).

15 February 1974; revised 24 May 1974

Scheinberg begins his comments by stating that the results of Evans *et al.* (*1*) are doubtful because the criteria of reversibility and equilibrium for the binding reaction were not met. He then launches into a discussion of L-6-D, the relevance of which I fail to see. No binding studies have ever been reported for this protein, and what data is available cannot be used to refute the results of Evans *et al.*

I agree that experiments at a lower *p*H would have been preferable, but our results indicated that copper was in equilibrium with metallothionein at pH 7.4 after 48 hours. We are currently reexamining copper binding to normal metallothionein and metallothionein from subjects with hepatolenticular degeneration under different experimental conditions.

If, as Scheinberg suggests, copper is bound to metallothionein in the cuprous form, our interpretation of the data is indeed incorrect, and I will take full responsibility for dubious experimental design and incorrect interpretation of results. However, before submitting to Scheinberg's refutation, I would like to see experimental evidence proving that copper is bound in the cuprous form. Scheinberg has stated that "it is *almost* certain that copper is bound as cuprous ions . . ." (italics mine) and referenced experiments demonstrating that copper is reduced by thiols. Scheinberg has overlooked the fact that we are dealing with a unique protein—a protein in which an average of three cysteinyl residues are involved in the binding of each metal atom (*2*). A complex involving three sulfhydryl groups, possibly another electron-pair donor, and a cupric ion would preclude oxidation-reduction and permit equilibrium to be obtained in a solution containing cupric ion. Indeed, cupric complexes with two cysteine molecules have been described (*3*). Thus, the presence of a thiol and a cupric ion does not necessarily dictate oxidation-reduction.

G. W. EVANS

Human Nutrition Laboratory, Agricultural Research Service, Grand Forks, North Dakota 58201

References

1. G. W. Evans, R. S. Dubois, K. M. Hambidge, *Science* **181**, 1175 (1973).
2. J. H. R. Kagi and B. L. Vallee, *J. Biol. Chem.* **236**, 2435 (1961); P. Pulido, J. H. R. Kagi, B. L. Vallee, *Biochemistry* **5**, 1768 (1966); J. H. R. Kagi, S. R. Himmelhoch, P. D. Whanger, J. L. Bethune, B. L. Vallee, *J. Biol. Chem.* **249**, 3537 (1974).
3. D. Cavallini, C. DeMarco, S. Dupre, G. Rotilio, *Arch. Biochem. Biophys.* **130**, 354 (1969).

24 June 1974

Subsidence of Venice: Predictive Difficulties

In explaining the subsidence of Venice, Gambolati *et al.* (*1*) have presented a very clear description of the importance of the consolidation of compressible soils. The mechanism they describe is a very reasonable theoretical formulation of the well-known consolidation theory of geotechnical engineering. Our purpose in this comment is to amplify the warning of Gambolati *et al.* in their concluding paragraph on possible inaccuracies in the predictions resulting from the lack of data.

It is very difficult to obtain a sample of undisturbed soil or rock at depth, and the accuracy of predictions for the time rate of settlement depends very heavily on the precision with which the properties of the materials can be measured either in the field or in the laboratory. When existing data are examined and the properties computed so as to provide a plausible fit to the already known history of settlement, very good agreement can be obtained. Unfortunately, when the calculations are extrapolated into the future, the fit can become quite precarious. For example, Fig. 1 is a summary of several predictions made for the subsidence at Long Beach, California, associated with oil field pumping. The solid line is the actual history of subsidence at the center of the bowl. The subsidence was stopped at 28 feet (8.5 m) when water was injected back into the field. The dashed lines are the predicted extrapolations made over a period of some 15 years by various well-qualified investigators, most of whom used the consolidation theory. It should be noted that all the predictions in Fig 1 were in error on the unconservative side, that is, too low. Similar difficulties have been experienced when investigators have tried to extrapolate the future behavior of other cases of subsidence due to pumping.

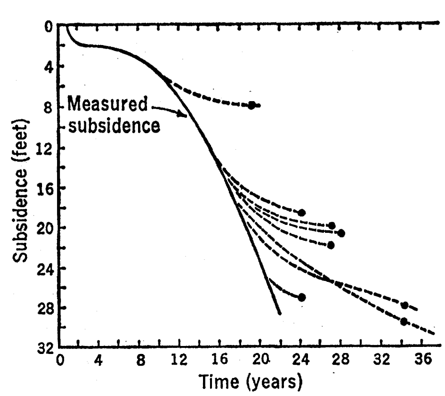

Fig. 1. Predictions made for the subsidence at Long Beach, California, associated with oil field pumping in the Wilmington oil fields.

Errors in predictions like those in Fig. 1 or those in figure 2 of (*1*) can arise from several sources. First, there is the above-mentioned difficulty of obtaining good samples of the soil. Second, details of stratification can strongly affect the time rate of settlement prediction, and such details are not easy to identify over the entire area of the settlement bowl. Third, fluid pressures are usually measured in the wells, where they must have the lowest values anywhere in the region. This leads to an underestimate of the overall fluid pressure distribution and distorts the interpretation of past history. A final cause of possible difficulty is the implicit assumption that the silt below 300 m is incompressible and does not contribute to the settlement. This assumption is questionable, and deformations in this material could throw the predictions off significantly. It would be interesting to know how Gambolati *et al.* have dealt with these problems.

For these reasons, the predictions shown in figure 2 of (*1*) may not be very accurate. Undoubtedly, the continued pumping of water from the wells in Venice will cause increased settlement as the result of the consolidation of the underlying soils, but exactly what shape this settlement curve will take is very difficult to predict. Furthermore, the prediction that stopping pumping will have a specific effect on the amount of settlement is very doubtful. This observation is in no way meant to suggest that the description of the problem, the numerical calculations, or the major conclusions of Gambolati *et al.* are incorrect. Rather it is meant to add emphasis to their own warning that the predictions should be viewed with great skepticism and to point out that in many cases predictions of future settlement have been much smaller than the actual settlement that subsequently occurred.

JOHN T. CHRISTIAN

Geotechnical Division, Stone & Webster Engineering Corporation, Boston, Massachusetts 02107

RONALD C. HIRSCHFELD

Geotechnical Engineers, Inc., Winchester, Massachusetts 01890

References

1. G. Gambolati, P. Gatto, R. A. Freeze, *Science* **183**, 849 (1974).

9 April 1974