

tions of river mollusk shells according to Keith and Anderson's hypothesis, existing radiocarbon measurements suggest that humus in living soils is rarely older than 3000 years and averages closer to 500 years (5, 6). Hence, if the Keith and Anderson hypothesis is valid, the humus they call on must come predominantly from fossil rather than living soils.

Several additional points are pertinent.

1) Since lakes receive the majority of their salts from the rivers that feed them, were it not for alteration through exchange with atmospheric CO₂, the carbon isotope composition of lake carbon would be expected to be similar to that of the supply river.

2) That a major portion of the dissolved carbon in river water is derived from the oxidation of humus in the water, after it has been transported from soils, represents a strong departure from the usual thinking on the subject.

3) From the chemical composition of many terrestrial waters, solution of carbonate rocks can be conclusively demonstrated to be a major source of dissolved carbon and an entirely adequate source of the observed C¹⁴ deficiency (3).

4) Incorporation of metabolic CO₂, derived from oxidation of the humus by the mollusk, cannot be called on as a means to enhance the humus contribution to the shells of these organisms over that of the dissolved CO₂ in the water, because subaqueous plants coexisting with the shells show the same C¹⁴ anomaly (3).

In conclusion, any relationship between the radiocarbon concentrations in soil humus and fresh water mollusks is almost certainly coincidental.

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References and Notes

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X-rays: Are There Cyclic Variations in Radiosensitivity?

It is encouraging to see the interesting work of Pizzarello and his co-workers (1) being pursued with another organism. However, there are several gaps in the information given us by Rugh and his collaborators (2) which make it difficult to evaluate their results. Most important, perhaps, is that the environmental conditions are not adequately specified.

"Standard laboratory conditions" are likely to vary considerably from one laboratory to the next, and one would like to know the temperature regime and exactly when the lights went on and off. In addition, it is of extreme importance to know how long the animals had been kept under these conditions before irradiation. When transferred to a new light regime, mammals often take many weeks to become fully entrained to the new cycle. If, for instance, a large colony of mice were transferred from constant light to a light-dark cycle, one would expect that even the running activity of the colony as a whole would be conspicuously arrhythmic for at least 8 to 10 days or until the majority of animals became entrained to the cycle. If they were irradiated during those 8 to 10 days one might expect results very like those obtained by Rugh *et al.*, even though a further experiment in which the animals were left in the light cycle for 2 or 3 weeks might show a pronounced cyclic variation in radiosensitivity.

As Halberg has elegantly demonstrated (3), there may be rhythms of susceptibility to different drugs in the same organism (mice) which have very different phase relationships to the external light cycle. It would be naive, in the light of this work, to assume that a rhythm of radiosensitivity in mice would have the same relation to the light-dark cycle as one in rats. By choosing 9 A.M. and 9 P.M., Rugh *et al.* could conceivably have picked two points of equal value on a curve of large amplitude. Irradiating at 6-hour intervals as in Rugh's Fig. 2 would, of course, define a sine wave, but a sharp peak of sensitivity might easily be missed. It is therefore most interesting that the strongest indications of a rhythm of radiosensitivity in Rugh's study appear in the data from irradiations at 6-hour intervals.

Although there is not sufficient space to do so fully here, the same general

kind of discussion might profitably be applied to the report of Straube (4) on the same topic. The 3-hour difference in photoperiod is only one of the four differences in protocol between his experiments and those of Pizzarello *et al.* listed by Straube. If rats do have rhythms of radiosensitivity, then surely, as in other rhythms of sensitivity described in the literature, the variation is not a simple matter of day versus night, but follows a curve of some particular shape. Of course at present the amplitude of the peak(s) of this hypothetical curve is unknown, as is its phase relative to an external light cycle. There are published data (5) showing that the phase of at least one animal rhythm is strongly dependent on photoperiod. There exists therefore the possibility that by using a different photoperiod Straube has shifted the phase of his rats' sensitive period (relative to that of Pizzarello's rats) and then missed this sensitive period by irradiating at the same times of day.

Finally, it seems completely unwarranted to criticize Pizzarello *et al.* on statistical grounds, as do Rugh and his collaborators. The sample size (40 animals, 10 each for 4 separate experiments) is not large but the data are so clearly significant by any statistical test one could rationally apply that the authors quite properly refrain from bothering the reader with such tests.

It is of course important that the work of Pizzarello *et al.* be repeated by other investigators and that, if the effect is confirmed, its generality be examined with due regard to its possible importance in human diagnosis and therapy. However, if rhythms of radiosensitivity exist in organisms, they probably have much in common with other physiological rhythms and, if one is to look for and study them, one must be aware of the techniques and precautions in general use in this field.

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