change with liquid water present in animal tissue in vivo would be at least as rapid as the exchange with water vapor we observed in our experiment. Our data thus indicate that a large fraction of the organically bonded hydrogen in whole mouse tissues cannot be controlled by the hydrogen isotopic composition of mouse food, since it must exchange with water present in the tissues both in vivo and postmortem. Our experiment does not address itself to an additional nondietary influence on animal D/H ratios—namely, the likely possibility that some of the nonexchangeable hydrogen in these tissues was derived from the water in body fluids when the proteins, lipids, and other compounds that make up the tissues were synthesized.

The data in Table 1 illustrate one other problem with the suggestion (*1*) that the D/H ratios of animals can be used to obtain information about their diets. The D/H ratios of the organically bonded hydrogen of animal tissues must depend on the relative abundances of the numerous chemical components that constitute them, since these components can have different D/H ratios (*4*). The difference of approximately 70 per mil we observed between the δD values (*6*) of brain and liver taken from the same mice almost certainly is caused by the higher concentration of lipids in brain as compared with liver (*7*), because lipids have more negative δD values than the other components of animal tissue (*4*). A difference of 70 per mil between the δD values of two animals living in the same habitat would be interpreted as evidence for different diets if the suggestion of Estep and Dabrowski (*1*) were followed, yet such an isotopic difference could merely be a reflection of differences in fat content of two organisms feeding on the same food sources.

In summary, we believe that the experiment of Estep and Dabrowski (*1*) does not show that the D/H ratios of the organically bonded hydrogen in animals are determined by the D/H ratios of their diets. We suggest that the hydrogen isotopic composition of an animal is controlled by a variety of factors, including its chemical composition as well as the D/H ratios of its food and water. We believe that further experimentation is needed to determine whether dietary information can be obtained from analysis of the isotopic ratios of animal hydrogen.

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$$\delta D = \left[\frac{(D/H)_{sample}}{(D/H)_{standard}} - 1\right] 10^3$$

the standard is standard mean ocean water (SMOW).
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Since the pioneering work of Schoenheimer (*1*) nearly 40 years ago, it has been generally accepted that "substances with 'labile' isotopes . . . cannot be employed for biological tracer experiments, as they will lose the marker immediately after ingestion, the heavy hydrogen being replaced by normal hydrogen of the aqueous body fluids. Hydrogen attached directly to carbon is generally stably bound." In control experiments, Estep and Hoering (*2, 3*) demonstrated that the organic hydrogen in the macromolecules in algae does not exchange extensively with water while in living cells or in intact but dormant cells. Even algae subjected to careful sonication did not exchange hydrogen isotopes. Only after the cellular material had been denatured by heating at 56°C for 24 hours was the cell hydrogen extensively exchanged with the hydrogen in water.

Estep and Hoering (*3*) showed that, when cultures of algae growing photosynthetically in water with a δD of −60 were transferred to an identical medium, where the δD of the water was changed to +60, the δD of the cells did not rapidly reflect the change in the δD of the water, although the algae went through four doublings in population each day for 4 days. Estep and Hoering postulated that the hydrogen is metabolized into the cells by way of a pool of a bound complex that does not exchange rapidly with cellular water.

Apparently, under some conditions, the organic hydrogen in cells is not as labile toward exchange with water as Schoenheimer's principles predict. DeNiro and Epstein's criticism (*4*) is based mainly on experiments that are vague and difficult to interpret. First, mouse food and tissues were exposed to water vapor above liquid water at an unspecified temperature. Temperatures considerably above the body temperature of the mouse will cause the rupturing of quaternary and possibly even tertiary hydrogen bonds. Second, the initial isotopic composition of the organic samples is not reported. Third, the liquid water was analyzed for its hydrogen isotopic composition, but the composition of the water vapors contacting the organic substances will be depleted in the heavy isotope. Fourth, the mouse tissues were homogenized and freeze-dried before the exchange experiment, and it is difficult to assess the extent of denaturation. It is difficult to compare the results of experiments of Estep and Dabrowski (*5*) carried out with living mice and snail tissues and of Estep and Hoering (*2, 3*), conducted on algal cells with those of DeNiro and Epstein in which homogenized and freeze-dried mouse tissue was used.

DeNiro and Epstein note (*4*) that "Estep and Hoering . . . observed an isotopic fractionation between some plants and the water in which they grew that was about as large as the difference Estep and Dabrowski . . . reported between the D/H ratios of the mouse tissues and the water the mice drank." They then hypothesize that "it is certainly possible that the fractionations that occur during the incorporation of hydrogen from water into the proteins, lipids, and other compounds of which organisms are comprised are of the same sense and magnitude in both plants and animals." The coincidence in an isotope fractionation of a similar magnitude in photosynthetic plants and heterotrophic organisms may be possible and would greatly compound the difficulty of interpretation. Estep and Hoering (*2, 3*) have shown, however, that, when algae are shifted from photosynthetic to heterotrophic growth, the isotopic composition of the algae shifts and becomes similar to that of the organic food source. In fact, when cells are grown photoheterotrophically (in light with glucose), the isotopic composition of the algae reflects both the organic substrate and photosynthetically fractionated material. If exchange of organically bonded hydrogen with cellular water were the controlling factor, the isotopic composition of the cells should be independent of the mode of growth.

At present, there is some uncertainty about whether the isotopic composition of the hydrogen in prey and predators can be used to follow food chains, but similar criticism may also be applied to the use of carbon or nitrogen isotopes in analogous studies. DeNiro and Epstein specifically criticize (*4*) that "the D/H ratios of the organically bonded hydrogen of animal tissues must depend on the relative abundances of the numerous chemical components that constitute them, since these components can have different D/H ratios." Biochemical fractions, that is, lipids, proteins, and carbohydrates, not only have different hydrogen isotope ratios but also different carbon and nitrogen isotope ratios (*6*, *7*). Different tissues from mice fed a single diet may have a difference of 5 per mil in their carbon isotopic compositions (*6*). The symmetry between the results found for snails and their known food source and the results for mice and their known food source indicates that the isotopic composition of the hydrogen in the diet is at least a very important factor controlling the hydrogen isotopic composition in the predator.

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Angiotensin II Binding Sites in Rat Brain

We here point out that the report by Landas *et al.* (*1*) does not represent the first direct evidence of angiotensin II receptors in the organum vasculosum of the lamina terminalis (OVLT) of the rat brain. Using a quantitative light microscope radioautographic approach we observed previously (*2*) that blood-borne ^{125}I-labeled angiotensin II binds to specific sites in all of the circumventricular organs of the brain, including the OVLT. The specificity of angiotensin II binding sites was established by means of competitive binding studies in vivo showing quantitatively that angiotensin II antagonists blocked the binding of ^{125}I-angiotensin II to the OVLT, whereas competition with a structurally dissimilar peptide was ineffective. We have also observed (*3*) that specific binding sites for blood-borne angiotensin II are concentrated within the neuropil about the capillary plexus of the OVLT. Landas *et al.* (*1*) observed that CSF-borne angiotensin II binds to sites along the ventricular surface of the brain adjacent to the OVLT. These combined observations provide evidence for the existence of two anatomically distinct populations of angiotensin II receptors in the OVLT. We bring these facts to light to reemphasize that topographic differences in circumventricular angiotensin II receptors may be the basis for differential effects of angiotensin II on brain function, particularly when angiotensin II is administered to the brain by anatomically different routes.

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Our report (*1*) appeared independently and in the same year as that of van Houten *et al.* (*2*). Both studies support the hypothesis that we proposed over 5 years ago, that the organum vasculosum lamina terminalis (OVLT) is a receptor site for angiotensin II (Ang II) with receptors on the blood side and on the ventricular side, whereas the subfornical organ contains receptors to blood-borne angiotensin II only (*3*). Their autoradiographic study, however, is not direct evidence of angiotensin II receptors but of angiotensin II binding, since they did not provide evidence of any biological response. In our study, we tested both the biological response and binding. Animals responded to intraventricularly administered fluorescein isothiocyanate-labeled angiotensin II by drinking. The brains were rapidly removed, frozen, and cut to reveal that the site of fluorescent binding was exclusively on the OVLT. In addition to this study, we have accumulated the following evidence to support the hypothesis. We showed that microiontophoretic application of angiotensin II excited cells in the OVLT of anesthetized rats (*4*) and in brain slices from unanesthetized rats (*5*). Blocking access to the OVLT by a ventricular plug abolished the response to intraventricularly administered angiotensin II (*6*) but not intravenously administered angiotensin II (*7*). Very low doses of angiotensin II applied directly to the OVLT produced drinking and pressor responses (*8*). In carefully dissected OVLT and subfornical organ tissue, we showed higher binding levels for angiotensin II in both organs compared to the cortex and an increased level of binding in the OVLT of hypertensive rats (*9*). Therefore, we can add the data of the autoradiography to the list of different results from other laboratories which supports the idea of the OVLT as a specialized receptor area for blood-borne and CSF-borne angiotensin II.

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Hydrogen Isotope Ratios of Mouse Tissues Are Influenced by a Variety of Factors Other Than Diet

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