containing saline alone. Then .05 ml of undiluted rabbit semen was added to each watch glass. Periodic observation revealed that the dispersal of the cumulus clot took 10 min in the saline solution, and ½–1 hr in the phosphorylated hesperidin. Therefore, the phosphorylated hesperidin used in the present study was undoubtedly potent as a hyaluronidase inhibitor, but its potency is relatively weak as compared with nitrated hyaluronic acid (*5*) in the inhibition of follicular cell dispersal and in the inhibition of the fertilizing capacity of spermatozoa.

Our knowledge of the actual function of sperm hyaluronidase in fertilization is as yet obscure, but we do know that one of its functions is the dispersal of follicular cells surrounding the egg. However, since it has been shown by several investigators that follicular cell dispersal is not a prerequisite of sperm penetration into the eggs (*6*), a hyaluronidase inhibitor would not necessarily be a fertilization inhibitor.

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The Natural Concentration of Deuterium in Honey[1]

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In the early 1930s, when heavy hydrogen excited the interest of scientists, extensive studies were carried out with the application of deuterium to biological systems.

The enthusiasm resulted in interesting findings by Hevesy, Schoenheimer, Rittenberg, Dole, Washburn, DeWitt-Stetten, and others, yet many problems were left unsolved and many phenomena unexplained.

It has been found that living organisms will tolerate only to a certain limit the exchange of the water in the body to the chemically identical deuterium oxide. Experiments on this problem resulted in the hypothesis that deuterium, having a different resonance, or exchange potential, from hydrogen toward certain bonds of intermediate metabolites, will interfere with the proper work of the donors or acceptors, as well as other enzyme systems essential for the maintenance of life. In accordance with this theory some of the data indicate that living tissues have a tendency to accumulate deuterium to a limited degree.

The appearance of deuterium in tissues can be easily explained by the uptake of natural water, which contains in general about 1 part of the isotope in 5000 parts (*1*) or, more precisely, 0.0147 mol% D (*2*). But further work is necessary to explain, for example, why the sap of a willow tree is significantly richer in deuterium and why the wood has an increment double that of sap (*1*). Since the vapor pressure of deuterium oxide is about 7% below that of water (*2*), it is possible that through transpiration plants are acting as a still and that by fractioned distillation of large quantities of water they might accumulate deuterium oxide in their tissues.

It is known that hydrogen of the OH-groups in glycogen or sugars can be exchanged for deuterium (*3*) if the latter is in abundance in the environment, but this process is slow (*4*) and would hardly account for the excess of deuterium in metabolites. A more likely theory is that deuterium enters into metabolic processes and becomes bound to carbon atoms (*5*); hence the increment as reported, for instance, in honey (*6*).

Little is known about the physiological effect of deuterium, but since it does interfere with metabolism and it is built into carbohydrate molecules, it is available to every cell or tissue through anabolic reactions. Beyond the known physiological action of deuterium, there might be a long-range effect reaching into the realm of genetics, or the theories of neoplasts.

Density measurements for the determination of the deuterium content were carried out with the falling-drop (*7, 8*) or the totally immersed float method (*9, 10*) or with the Cartesian diver. These methods are very sensitive, yet from many points of view it is more convenient to use a mass spectrometer (*11*).

It was of interest to repeat the experiments with honey, extending them to separate determinations of the deuterium ratio in the moisture and the sugars of honey. The material used was a *i95i* buckwheat honey from the Finger Lakes region, with a moisture content of 17.9%. The following samples were analyzed:

Sample M: The somewhat crystallized honey was dehydrated at room temperature, and the moisture trapped in a cooling mixture of acetone–solid carbon dioxide. The condensed liquid was extracted with ether to eliminate volatile metabolites, then the total amount was redistilled to avoid fractionation of deuterium oxide.

Sample M_2: To eliminate crystal water, the dry honey was heated slowly under normal pressure until the first signs of caramelization. This residual water was condensed and passed through a combustion tube.

Sample S: The slightly caramelized honey was combusted with oxygen and a platinum sponge. The condensed water was recombusted to eliminate all traces of organic matter.

Sample D: The crystals of the honey, mainly dextrose, were separated by vacuum filtration and washed with absolute methyl alcohol.

Sample L: The honey which passed through the Buchner funnel was dried *in vacuo*. It contained 36% more levulose than dextrose. Samples D and L were combusted as described for Sample S.

For the measurements in the mass spectrometer,

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about 0.1 ml of the sample was placed in a small platinum container in a combustion oven. The vapor passed through zinc, heated just below its melting point. The resulting hydrogen and deuterium were accumulated in a sampler and fed through a manifold into the tube of the spectrometer. The resulting data on mass 3/mass 2—that is HD/H_2—were converted into the excess in mole % deuterium. The averages of the results are shown in Table 1. The results indicate

TABLE 1

Sample	Excess (mole % D)	Increment (% D)
M	0.0025	17
M_2	.0025	17
S	.0041	28
D	.0031	21
L	.0044	30
Wax	0.0028	19

that the natural abundance of deuterium in honey, as determined through densimetric measurements by Dole (*6*), seems to be verified by these mass spectrometer data. The error in the measurements with the mass spectrometer is in general below ± 5%.

The differences in the various samples are distinct, and, compared with the average of deuterium in rain and lake water, as given by Bleakney and others (*12, 2*), they are significant.

The values obtained in this table are based upon our standard containing 0.0148 mole% D, and we can assume that buckwheat plants in the Finger Lakes region have taken up water with the same average deuterium content.

The increment of the isotope deuterium in Sample M might indicate that the moisture in the nectar of flowers is coming partially from anabolic degradation of sugars, and is probably enriched through repetitive processes.

The fact that honey contains more levulose than dextrose, and that the levulose has in this sample about two times higher deuterium content, could point to a specific deuterium affinity of the invertase in the stomach of the bees, which could build deuterium atoms into sugar molecules at some point in the hydrolytic process. There are a few indications in the literature that deuterium has an influence on enzymatic systems (*13*): inhibitory action at higher concentrations (*14*), stimulating at low concentrations (*15*), even raising the resistance of certain bacteria toward strong disinfectants (*16*).

It should be mentioned that the dispersion coefficient—namely, $\frac{\text{mass 3/mass 2 in sugar}}{\text{mass 3/mass 2 in water}}$—is quite negligible by such low concentrations as it is present in living tissues, and the dispersion coefficient of fructose, which is about 10% higher than that of glucose (*17*), cannot account for the excess of deuterium as found in our experiments.

These data and the preliminary conclusions drawn need, of course, further work and confirmation. Besides the repetition of above experiments with other honey samples it would be interesting to have the data on the water from the geographical micro-unit where the honey was gathered; it would also be important to feed plants with excess heavy water and analyze the nectar for its deuterium content, or feed the bees with deuterium oxide and determine the deuterium in the sugars of the honey produced by them. Beyond the apicultural significance of this question it is enormously interesting for our general concept of biochemical reactions.

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Competitive Action of Isonicotinic Acid Hydrazide and Pyridoxal in the Amino Acid Decarboxylation of *Escherichia coli*

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We have previously shown that the inhibitory effect of isonicotinic acid hydrazide (INAH) on the growth of *Escherichia coli* significantly decreases in broth media (more than 10 mg/ml), compared with that of INAH (0.6–1.2 mg/ml), in a synthetic medium (Anderson's *M*-9 medium) (*1, 2*).

Thus it is possible to postulate the existence of certain substances that may be contained in broth and that are able to inhibit the action of INAH on the growth of *E. coli*. In further investigations of these postulated substances, we found that one of them can, to some extent, be replaced by pyridoxine hydrochloride (*1, 3, 4*); hence, it may be suggested that there is an intimate connection between INAH and pyriodoxine and its derivatives.

It has been generally agreed that pyridoxine derivatives play an important part in some enzyme systems of *E. coli*—i.e., decarboxylase (*5, 6*), tryptophanase

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