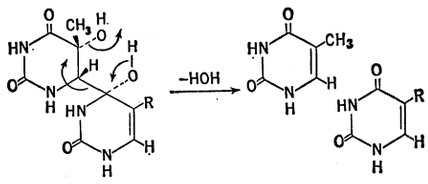
drogen bonds, which are illustrated by the light lines in the stereoconfiguration of the molecule shown in Fig. 2.

The reversion of PT_1 to thymine has been observed under various conditions (*6*) and conceivably occurs through the following mechanism (*7*). Thus, this diol structure is of particular interest in



considering reactivation processes in photobiology. If such a reaction involves cytosine as one of its bases, deamination may occur, resulting in the transformation of a cytosine to a uracil moiety (*7*). Such a change should be examined in relation to biological mutations induced by light. The x-ray data indicate that both OH groups are *trans* to the hydrogen on C-26. This configuration favors *trans* elimination of HOH rather than the rearrangement shown above and explains why the reversion of PT_1 to thymine is not a major reaction. Isomeric photoproducts with an all *cis* adduct linkage may also form. Such a configuration, that is, one in which both OH groups are *cis* to the CH, would favor the above rearrangement, and such a reversion to the bases might occur at rather mild acidic or basic conditions. Therefore, even if adducts of this type were present, they could not be detected by assay of the acid hydrolyzates of nucleic acids irradiated with ultraviolet light, a method generally used to analyze photoproducts. Even if undetectable chemically, however, their presence may be manifested by certain biological effects in systems in vivo.

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Deuterium: Natural Variations Used as a Biological Tracer

Abstract. *The suggestion is made that isotope tracing be carried out by monitoring the natural variations in deuterium concentrations. As an example, the natural variations in deuterium concentrations between food and water collected in Illinois and food and water collected in Colorado were used to determine the residence time of water in the blood and urine of rats. We observed not only a 5½-day turnover time of water in the blood and urine, but also evidence for the influx of water vapor from the atmosphere through the lungs into the blood.*

The use of deuterium as D_2O, heavy water, in isotope tracing is not new (*1*). The results indicate that rats cannot survive replacement of more than about one-third of the body water by D_2O. As the concentration of D_2O in the serum of rats approaches 20 percent, the rats show signs of hyperirritability,

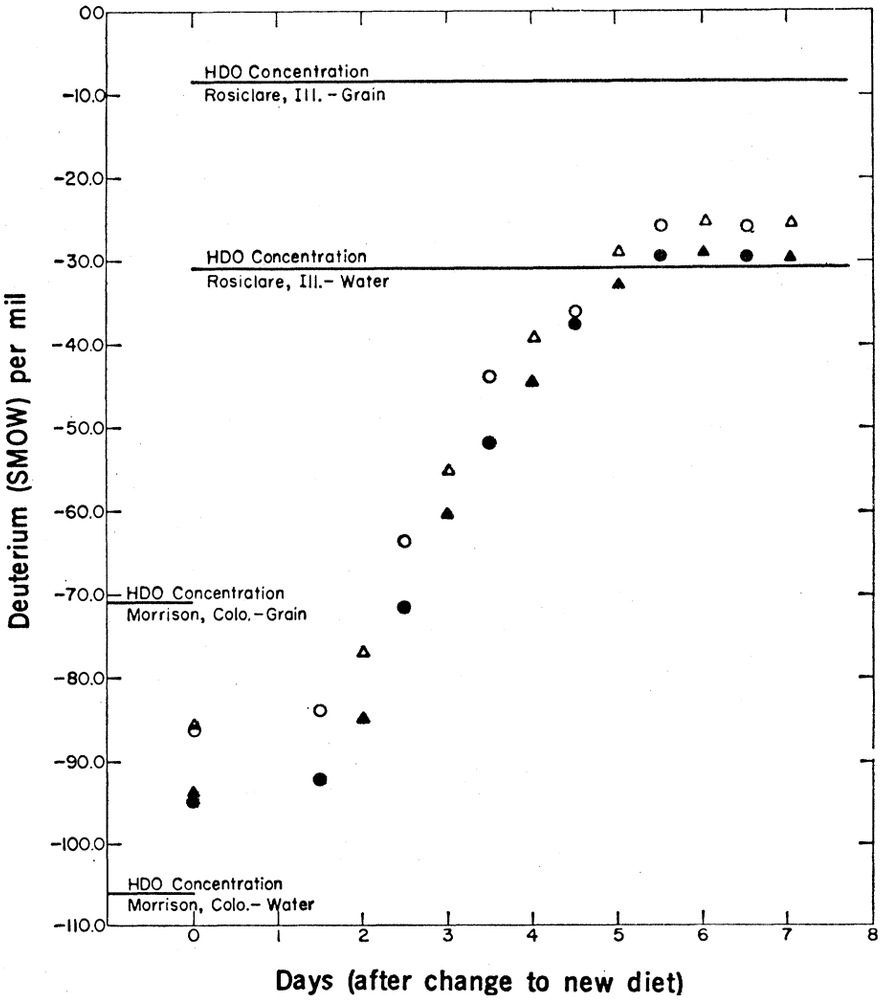

Fig. 1. Relative deuterium concentration in deviation per mil from SMOW standard of blood and urine of rats plotted as a function of time after their diet was changed from one low in deuterium to one relatively enriched in deuterium. ○, blood, rat No. 1; ●, urine, rat No. 1; △, blood, rat No. 2; ▲, urine, rat No. 2.

they become prone to convulsions, their liver and adrenal glands increase in size, and they become anemic. In some animals the count of red blood cells decreases to less than 20 percent of the normal value.

This study, however, is concerned with the feasibility of using deuterium at the natural abundance level, which is on the order of 0.015 mole percent D (the ratio of D to H = 150 parts per million). Animals living in two sections of the country that differ in the amount of deuterium naturally present in the groundwater and existing on a diet of water from this environment along with grain and other foodstuffs grown there can be expected to have different amounts of deuterium in their body fluids. The reason for the difference in the deuterium content of natural waters was given by Kirshenbaum (*2*) as follows:

> It is not unreasonable to expect that fractionation may occur as precipitation of water vapor from the air occurs. The first fraction precipitating may be higher in deuterium owing to the difference in vapor pressure of H_2O and HDO. Therefore as the winds rise to go over the mountains and much precipitation takes place, the effect may be that of the residual water having low deuterium content. Consequently the waters which receive the last rains from these winds would have a low deuterium content.

A large number of deuterium analyses on natural waters have shown variations as large as 50 percent (*3*). Since variations as small as 0.1 percent can be measured, deuterium should be a sensitive natural tracer.

Rats were used in this study because of their hardiness and because they are resistant to possible infection caused by the repeated taking of blood. Diets were chosen from two areas that have a marked difference in deuterium content in both grain and water. For the lower concentration of deuterium samples from Morrison, Colorado, were used, and for the higher concentration samples from Rosiclare, Illinois, were used.

The two rats used were first placed on the diet of water and wheat collected near Morrison, Colorado. This diet was depleted in deuterium content since the water was −106 per mil and the grain was −71 per mil [relative to SMOW (standard mean ocean water) standard]. The rats were kept on this diet for approximately 2 weeks to ensure that equilibrium had been established between the deuterium content of this diet and that of the blood and urine of the rats. Katz found that approximately 6 days were required for equilibrium to be established between the deuterium content of the rat urine and that of the diet when the rats were given water of high concentrations of D_2O (*1*). Blood and urine samples were taken for two consecutive days to ensure that equilibrium had been reached. The diet was then changed to the wheat and water from Rosiclare, Illinois. This diet was significantly higher in deuterium content (H_2O = −31 per mil, wheat = −9 per mil). Blood and urine samples were collected daily from both rats until equilibrium had been established with the new diet.

The water was analyzed by the method of Friedman (*4*). In this method hydrogen is liberated from the water when it passes over hot uranium metal. The relative deuterium concentration of the hydrogen is then analyzed on a double-collecting mass spectrometer.

Wheat was analyzed by drying one kernel in a vacuum at room temperature for 20 minutes. It was then heated to above 250°C and the water was collected in a trap cooled with dry ice. The water was then analyzed as discussed above. Repeated analyses gave similar results, as long as the temperature was kept above 250°C.

The rat was placed in a Büchner funnel 7 inches (17.8 cm) in diameter when its urine was collected. Covering the rat was a large funnel-shaped piece of tin with a small air hole at the top. After micturition the urine was sealed in small glass capillaries as in the analysis of water, and these samples were analyzed in the same way as the water samples.

Blood was obtained from the rat by cutting the tip of the tail with sterile scissors. The first drop was discarded and the samples were taken by means of small capillaries directly from the tail. The ends of the capillaries were immediately sealed in a flame. The blood-filled capillaries were broken in a vacuum system and the residue was heated to 300°C or more to extract the water, which was then analyzed as described above.

Rat No. 1 weighed 250.7 g when its diet was changed from water and wheat of Morrison, Colorado, to water and wheat of Rosiclare, Illinois. It ate 159.6 g of wheat, drank 296 ml of water, and gained 25.7 g during the next 9 days. Rat No. 2 weighed 269.2 g when its diet was changed. It ate 239.5 g of grain, drank 401 ml of water, and gained 31.0 g during the next 9 days. The experimental rats apparently ate and drank normally.

As can be seen from Fig. 1, the deuterium content of the blood and urine of the rats equilibrated to a new deuterium concentration after 5½ days of food and water intake. During this time the rat had ingested an amount of water from food and drink about equal to three times the weight of its total body water. The concentration of deuterium in the blood and urine did not change appreciably for about 36 hours after the change in diet, and then it changed rapidly until equilibrium was reached in 5½ days.

At the beginning of the experiment the blood of the rats was enriched in deuterium by 8 per mil as compared to the urine, and enriched by 20 per mil as compared to the Colorado water. At the end of the experiment the blood was enriched by only 4 per mil as compared to the urine, and by 5 per mil as compared to the Illinois water. These relative depletions, particularly in the blood but also in the urine at the completion of the experiment, can be explained by the fact that the animals were continually breathing deuterium-depleted water vapor (near Morrison, Colorado) and that the composition of this water vapor significantly changed the deuterium content of the blood and the urine. From these data it would appear that appreciable influx of water vapor occurs in the rat lung, and possibly through the skin as well. Although the major flux of water through the lungs is directed outward to the atmosphere, a smaller counterflux of water vapor from the atmosphere into the body fluids by way of the lungs must occur.

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