and heavy water molecules diffuse through the root membranes in the proportions in which they are present in the water, that is, no appreciable isotopic fractionation occurs in this process.

THE ISOTOPIC COMPOSITION OF THE SAP WATER AND OF THE COMBINED HYDROGEN OF A WILLOW TREE

Although no isotopic fractionation occurs during the ingestion of water through the roots of the growing plant, there remains the possibility that an isotopic selection may take place within the plant as a result of evaporation or of photosynthesis. In order to obtain information on this question the following experiments were made.

In the summer of 1933 a quantity of branches and leaves were secured from a young willow tree (*Salix nigra*) growing on the bank of a small stream. The green branches were wrapped for transportation in a waterproof blanket and taken to the laboratory. The next day they were placed in a large iron pipe and heated to 150° C. in a current of dry nitrogen. The expelled sap water was condensed and collected. The temperature was then raised to the ignition point and the dry wood was burned in a current of dry oxygen. The water resulting from this combustion was also condensed and collected.

The organic matter in both samples of water was destroyed by wet combustion with alkaline permanganate, and the density of the water, after careful purification, was measured, with the following results:

Sap water (A) Δ[a], ppm ($\pm$ 1)	Water from the combined hydrogen (B) Δ[a], ppm ($\pm$ 1)
2.8	5.5
2.8	5.2
—	—
2.8	5.4

[a] Δ = Excess density as compared with "normal water," in parts per million. The source of the normal water used for comparison was the Potomac River.

Both samples of water were therefore heavier than normal by small but significant amounts.

In order to determine the nature of the isotopic change which had occurred, the following experiments were made, in accordance with the methods of isotopic analysis first employed by G. N. Lewis.[3] A colloidal solution of platinum containing platinum black in suspension was prepared in the water of sample B, and normal hydrogen gas was bubbled through it for 24 hours.[4] After purification its density was found to be unchanged ($\Delta = 6.3 \pm 1$). The reaction with hydrogen gas under these conditions is apparently not sufficiently rapid to completely normalize water with respect to hydrogen.

In order to make sure that the oxygen of the water had the normal isotopic composition, the sample was then given an extended treatment with dry CO_2, followed by distillation from a large excess of solid K_2CO_3. Again no change in density occurred ($\Delta = 6.4 \pm 1$).

Finally the water was saturated (approximately) with dry gaseous NH_3 and then desaturated, the process being repeated six times. This treatment lowered the excess density to $\Delta = 3.1$, a drop of 3 ppm, thus showing definitely that the heavy water contained a higher percentage of the heavy isotope of hydrogen (deuterium) than normal water.

CONCLUSIONS

During the process of the synthesis of organic compounds by a growing willow tree, an isotopic fractionation of hydrogen occurs in the direction of a preferential selection of the heavier isotope with the result that the sap and the combined hydrogen of the woody parts of the plant both yield heavy water. Whether this preferential selection of deuterium is beneficial, detrimental or innocuous to the organism can be determined only by further experimentation.

If the phenomenon is general in the vegetable kingdom, it is probable that many of the organic compounds which we obtain from plants (*e.g.*, oils, carbohydrates) do not, in their native state, have normal isotopic composition. Many of them (those containing ionizable hydrogen) would, however, be normalized, at least partially, if in the process of extraction or purification they are treated with water; as in the case of the starches and sugars, for example.

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[3] *Jour. Am. Chem. Soc.*, 55: 3502, 1933, and *Jour. Chem. Phys.*, 1: 343, 1933.

[4] *Cf.* J. Horiuti and M. Polanyi, *Nature*, 32: 819, 1933.

HEREDITARY VARIATIONS IN THE BLOOD CYTOLOGY OF NORMAL RABBITS

STUDIES in this laboratory have demonstrated wide variations in the blood-cell formulae of normal rabbits comparable in magnitude with variations in coat color, size, body and organ weights, and other constitutional factors. Except for minor seasonal fluctuations these differing blood-cell formulae were found to be fairly stable during conditions of health and vigor and to be closely related to the natural resistance of the rabbit host to several experimental and spontaneous diseases. It seemed quite likely, therefore, that the differences in the blood formulae were largely inherited differences, just as the color of the

[5] The death of Dr. Washburn occurred on February 6.

coat, the blood groups,[1,2] abnormalities in the skeletal and cranial bones, etc. To substantiate this point the blood-cell formulae of standard varieties of rabbits were studied. In this study there were 146 adult male rabbits from 8 standard breeds, distributed as follows: 24 Havana, 19 Himalayan, 14 Belgian, 24 English, 16 Polish, 18 Dutch, 18 Beveren and 13 Rex animals. These breeds have been propagated in pure line in this laboratory from 3 to 8 years. The animals used were comparable as to age (average age 8.1 months) and physical condition and were examined in large groups diversified as to breed. Uniform diet and housing conditions were maintained. An average of 3.3 hematological observations were made on each individual rabbit distributed over a period of 2.1 weeks, including 4.0 red cell, platelet and hemoglobin estimations, 5.4 total and 6.3 differential white cell counts. The mean blood level for each cytological factor was computed. A statistical analysis of the material revealed that the blood cytology of individuals of the same breed was more alike than the blood cytology of individuals of different breeds. It was apparent that characteristic and typical blood formulae existed for each variety of standard breed rabbits studied and that the standard breeds in our laboratory could be identified on this basis. The chances of this being due to a random association of circumstances are remote, since for 11 of 14 blood factors the variance between breeds was significantly greater than the variance within breeds. (Red blood cells, $z = 0.92$, $P. = 0.01-$; hemoglobin, $z = 0.95$, $P. = 0.01-$; platelets, $z = 0.70$, $P. = 0.05-$; white blood cells, $z = 0.74$, $P. = 0.05-$; neutrophiles per cmm., $z = 0.10$; basophiles per cmm., $z = 1.06$, $P. = 0.01-$; eosinophiles per cmm., $z = 0.61$, $P. = 0.05-$; lymphocytes per cmm., $z = 1.17$, $P. = 0.01-$; monocytes per cmm., $z = 0.34$; neutrophiles in per cent., $z = 0.88$, $P. = 0.01-$; basophiles in per cent., $z = 1.12$, $P. = 0.01-$; eosinophiles in per cent., $z = 0.67$, $P. = 0.05-$; lymphocytes in per cent., $z = 1.07$, $P. = 0.01-$; monocytes in per cent., $z = 0.07$). The most striking variations between the eight breeds were in the values for the basophiles, lymphocytes, red blood cells and the hemoglobin. It is interesting and perhaps significant that this should be the case, since these latter factors were found to be reliable indices of the natural resistance of rabbits to inoculation with a transmissible malignant tumor or with the spirochete of syphilis. The lymphocytes were found to vary from a level of 3,780 per cmm. for the Polish breed to levels of 1,810 and 2,030 per cmm. for the English and Havana; the basophiles from 680 to 690 per cmm. in the Belgian and Beveren to 320 and 360 per cmm. for the Polish, Havana and Dutch; the red blood cells from 5,730,000 per cmm. in the Havana to 4,870,000 in the Rex; and the hemoglobin from 73.4 per cent. and 73.9 per cent. in the Havana and Polish to 68.1 per cent. in the English and Beveren and 63.6 per cent. in the Rex. In contrast to this wide variation, no significant differences between the breeds were found for the neutrophiles per cmm. or for the monocytes, either per cmm. or in per cent. The Belgian, English, Polish, Beveren and Rex breeds had mean neutrophile levels of 3,760, 3,720, 3,750, 3,740 and 3,770 per cmm., respectively. Not only were typical blood formulae found for each breed, but closely related breeds, such as the Belgians and the English, and the Havana and the Dutch had similar blood formulae, and relatively unrelated breeds had widely different blood formulae. In addition, if the breeds were grouped according to weight, that is, heavy and light, their respective blood formulae were likewise grouped, the heavier breeds having significantly higher total white blood cells and higher basophiles and monocytes per cmm. and in per cent., and significantly lower hemoglobin and red blood cells than the smaller and lighter breeds. The animals of some breeds were entirely uniform for certain blood cell factors, while the animals of other breeds were heterogeneous for the same factors. In breeds where heterogeneity existed for a given factor the animals in which the factor was high were more closely related than those animals in which the factor was low and *vice versa*. It was concluded, therefore, that the differences in the blood formulae among normal rabbits are largely inherited differences and studies on the transmission of such characters are now being made.

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