neutral red in dilute solutions are also useful for bringing living amoebae and cysts into contrast with the associated fecal debris.

The proposed staining solution is methyl alcohol saturated with methylene blue. This combines and surpasses the virtues of iodine and eosin solutions. If a small amount of feces is emulsified in a drop of water, an equal-sized drop of this stain mixed with the emulsion and a cover glass placed on the preparation, cysts and motile forms of amoebae will stand out as clear refractile bodies in the dark blue field. All fecal remains, with the exception of certain crystals, are stained dark blue and are thereby merged with the rest of the blue field. The whole preparation may be searched with the low power objective in a very short time. Examination with the 4 mm or oil-immersion lens will show the nuclear chromatin of amoebae and cysts to be selectively stained with the methylene blue. Chromatin beads about the nuclear membrane and karyosomes appear as distinctly as in haematoxylin stains of fixed preparations. The only precautions to be observed are to reduce the amount of light and to make thin preparations; either use small drops of fecal emulsion and staining solution, or use large coverslips to spread the mixture over a larger area.

Trophozoites are rounded up by contact with the methyl alcohol, but the nuclei may be seen distinctly, even in the presence of much ingested material. Frequently cultures of intestinal amoebae do not develop cysts, so that the diagnosis must be based on active forms. If these have ingested much particulate matter, especially starch particles, iodine is practically useless for staining the nucleus.

Castor oil and mineral oil droplets in feces are stained a light greenish-blue by this stain and do not have the clear refractile appearance of cysts.

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SPECIAL ARTICLES

THE ISOTOPIC FRACTIONATION OF WATER BY PHYSIOLOGICAL PROCESSES[1,2]

ABSTRACT

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 this plant both yield heavy water. No isotopic fractionation occurs during the passage of the water into the tree through the root membranes.

(1) INTRODUCTION

During the growth of plants, water is imbibed through the roots, and hydrogen from this water is then utilized by the plant in the photosynthesis of organic compounds. In both of these processes there exists the possibility of an isotopic fractionation, that is, the possibility of a selective action of the organism with respect to the light and heavy isotopes of hydrogen. The purpose of this investigation was to determine whether any such selective action occurs.

(2) PERMEABILITY OF ROOT MEMBRANES TO LIGHT AND HEAVY WATER

In August, 1932, an experiment was started for the purpose of ascertaining whether an isotopic fractionation of water takes place during osmosis through the roots of growing plants. In this experiment cow peas were first employed. The plants were wax-sealed through holes in the tight cover of a large jar containing 30 liters of tap water. Nutrient salts and oxygen by aeration were supplied as needed.

After about three months, during which time 12 liters of water had been evaporated, the cow peas succumbed to an attack by aphids and were replaced by plants of Indian corn. When these had consumed one liter of water (1 month), they in turn perished during a cold winter's night and were replaced by rooted twigs of the weeping willow and the experiment continued during the winter. By April 18 only 225 cm^3 of the original 30 liters of water remained. After careful purification, the density of this residual water was measured and found to be normal (± 1 ppm).

The experiment was then repeated, this time entirely with growing willows. This was in the summer of 1933 and transpiration was much more rapid than in the first experiment. Again the residual water from 30 liters was found to have normal density (± 1 ppm).

In a third experiment a sample of heavy water (sp. gr. 1.000,053) was reduced from 370 to 165 cm^3 by transpiration through willow, and the residual water was found to be unchanged in density (± 1 ppm).

From these experiments it is evident that the light

[1] Publication approved by the Director of the Bureau of Standards, U. S. Department of Commerce.

[2] Most of the experimental results presented in this paper, together with a description of the methods employed in purifying the water and in measuring its density, will be given in a forthcoming publication,—*Bur. Standards Jour. Research*, 12, 1934.

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.