atmospheric oxygen.[1] The increase amounted to 2.2 ppm for the oxygen tested from a certain commercial cylinder. At the same time that the measurements which demonstrated this difference were being made, Klar and Krauss[2] announced the discovery that the oxygen escaping with the nitrogen at the top of a column for fractionating liquid air is lighter than atmospheric oxygen and that the last portion of oxygen left after the fractionation of a charge of liquid air is heavier than normal. Their discovery has accordingly been confirmed, and in addition the magnitude of the difference to be expected in commercial oxygen from liquefied air has been established.

In a recent article in this journal,[3] it was reported that the water obtained from the sap of a young willow tree was heavier than normal by 2.9 ppm, and the water obtained by burning the dry wood in a current of dry oxygen was heavier than normal by 5.4 ppm. No explanation of the difference of 2.6 ppm between these values was offered at the time, but it is now clear that this increase was contributed by the commercial oxygen used in the combustion. It was also reported that the density difference of 5.4 was reduced to 3.1 ppm by repeated saturation and desaturation with dry gaseous ammonia. It is now clear that no further reduction in density difference was possible by this method because the remaining difference was contributed by the commercial oxygen. This additional information, however, does not change but rather serves to clarify the results and conclusions of the previous article.

EDGAR R. SMITH

BUREAU OF STANDARDS,
WASHINGTON, D. C.

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[1] E. R. Smith, *J. Chem. Physics*, 2: 298, 1934.

[2] R. Klar and A. Krauss, *Naturwissenschaften*, 22: 119, 1934.

[3] E. W. Washburn and E. R. Smith, SCIENCE, 79: 188, 1934.

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## ISOTOPIC $CO_2$ AND $O_2$ IN PLANTS?

WASHBURN and Smith report that the sap and combined $H_2$ in the wooden part of a growing willow tree yield heavy water. There appears to be a preferential selection of heavy $H_2$ isotopes in the process of synthesis of organic compounds.

Barnes observed the inactivation "in vivo" of certain enzymes in dilute heavy water. Barnes, Richards and Meyer conclude that high concentration heavy water has a lethal effect on plants while dilute heavy water shows a stimulating action.

It appears from such work that the water absorbed by plants for their physiological processes probably differs isotopically from the water lost during such processes.

This would suggest that $CO_2$ absorbed during the photosynthetic process and the $O_2$ absorbed during the respiratory process differ isotopically from the $O_2$ rejected during the photosynthetic process and the $CO_2$ rejected during the respiratory process.

ANDREW MOLDAVAN

MONTREAL, CANADA

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## THE CONTROVERSY CONCERNING THE PHYSIOLOGICAL EFFECT OF TRIHYDROL IN LIQUID WATER

A NOTE by Ellis and Sorge[1] reported that recently condensed water and water from freshly prepared ice showed similar infra-red absorption curves. The experiments were intended to throw light on the biological effects of ice water and steam water, but no mention is made of similar tests both positive and negative. There is no doubt concerning the physiological importance of trihydrol as an equilibrium constituent of water. As Duclaux[2] points out (from analogy with $H_2S$, etc.), water should boil about 100° below zero if it were not composed of aggregates. Properties which render water suitable as a life medium are due to its content of liquid ice, *i.e.*, the high specific heat is the heat of dissociation of trihydrol.

The estimates of the equilibrium concentration of hydrols are remarkably similar (*cf.* Whiting,[3] Sutherland,[4] Richards and Chadwell,[5] Rao[6]). Ice water has a favorable effect on the longevity of *Spirogyra* and stimulates cell division in *Euglena*,[7] and Harvey[8] has recently shown that the addition of ice water to sea water stimulates the growth of *Nitzschia*. It is possible that the equilibrium concentration of hydrols in ice water is not instantaneous, for experiments of Howard T. Barnes[9] indicate a lag in the polymer equilibrium during the exhaustion of the ice-forming power of water. In a typical experiment in a large water tank (−10° C.) the first hour yielded 8 pounds of ice, the second hour six pounds, the third hour five pounds, the fourth hour four and one half pounds, etc., until the ice-forming power of the water was practically exhausted. Other experiments[10] have shown that water from ice consisting of large crystals, warmed to 10° C. and placed in Dewar flasks at −5° C., will freeze more rapidly than recently condensed water under the same conditions. According to the colloidal theory, ice water freezes more rapidly

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[1] Ellis and Sorge, SCIENCE, 79: 370, 1934.

[2] Duclaux, *Rev. gen. Sci.*, 23: 881, 1912.

[3] Whiting, "A New Theory of Cohesion Applied to the Thermodynamics of Liquids and Solids," Cambridge, Mass., 1884.

[4] Sutherland, *Phil. Mag.*, 50: 460.

[5] Richards and Chadwell, *Jour. Am. Chem. Soc.*, 47: 2283, 1925.

[6] Rao, *Nature*, 132: 480, 1933.

[7] Barnes and Jahn, *Proc. Nat. Acad. Sci.*, 19: 638, 1933.

[8] Harvey, *Jour. Mar. Biol. Assoc.*, 19: 253, 1933.

[9] H. T. Barnes, *Sci. Monthly*, 29: 289, 1929.

[10] Barnes and Jahn, *ibid.*

because it takes less time to reach the saturation point of trihydrol. Bancroft and Gould[11] object that water has not a variable freezing point like acetaldoxime, but we refer to the *time* required for freezing.

Wills and Boeker[12] describe a hysteresis in the diamagnetic susceptibility of water, depending on its thermal history. This is not due to the apparatus[13] used, since other substances (carbon tetrachloride, toluene) do not show the lag. Some diamagnetic methods,[14] however, do not demonstrate the polymer hysteresis. Some years ago Baker[15] suggested that association takes place more slowly for liquids than for gases, *i.e.,* water heated at 80° C. for 48 hours (in presence of a catalyst) may exhibit an altered vapor pressure which persists for days.

Ellis and Sorge do not mention negative evidence supporting their own results. Menzies[16] tested my trihydrol hypothesis by comparing the vapor pressure of boiled (not steam) water and water from freshly prepared ice (fine needle crystals) by means of a water manometer. No appreciable vapor pressure difference was detected by this method, but Menzies used a small sample of freshly frozen water and boiled water, whereas the best biological results are found in water from aged block ice (large crystals) as compared to freshly *condensed* water. La Mer and Miller[17] suggest that Menzies' method was not sufficiently sensitive to detect small differences in polymers, although they find no difference in the refractive index of distilled water of different ages. Menzies expected a greater vapor pressure difference near 0° C. than at 25° C. in spite of the fact that 0° C. is the triple point at which the vapor pressure of ice (almost 100 per cent. trihydrol) and water (37 per cent. trihydrol) are the same (*i.e.,* both 4.579). Ice itself evaporates as a solid vapor.[18] Menzies[19] previously claimed that water vapor contains no polymers, but this was corrected by Maass and Mennie.[20] Vapor pressure may indicate activity of water molecules at the surface, but it fails to indicate bound[21] water demonstrated by other methods.[22]

Like Menzies, Ellis and Sorge use water from a small sample of freshly prepared ice, but x-ray studies[23] have shown that such samples do not yield single crystals with well-developed faces for which commercial block ice must be used. Plyler[24] states that the best crystals for infra-red studies are obtained by aging commercial block ice for two months (slightly below 0° C.) in which 5-inch crystals develop. The growth of ice crystals with age has not been explained, but Altberg and Troschin[25] have published beautiful photographs of single ice crystals half a meter in size found in caverns in the Ural Mountains. According to Plyler, the infra-red absorption of water and ice are similar, for both have a dielectric constant of about 80 for long waves. Ice has the corresponding infra-red bands of liquid water, but they are shifted slightly toward the longer wavelengths. Now, according to Sutherland, water at 0° contains 37 per cent. trihydrol and ice is almost 100 per cent. trihydrol, yet the differences in most of the infra-red bands are only a few units in the second decimal place, so that a 10 per cent. polymer difference in ice water would not be clearly indicated in Sorge and Ellis' method. Moreover, in one case Plyler found the bands of water and ice the same, due to a water film in the latter. Also, the temperature shift[26] in the infra-red bands of liquid water is much smaller than the polymer change. Ellis and Sorge used ice water at 21° C., while in our biological experiments the ice water is added at 10° C. Temporary exposure to cold water (high polymerization) may enable the cells to bind trihydrol, since Kolkmeijer and Favejee[27] find that the bound water in emulsoid sols is arranged in the ice lattice.

Bancroft and Gould suggest that our results may be due to an unsuspected impurity or to the presence of the hydrogen isotope. The impurity explanation is improbable, since clear block ice yields dust-free conductivity water and there is no significant pH difference indicating a different $CO_2$ content in the water samples (which were bubbled with $O_2$ in some cases). Also Harvey obtained the biological effect, under certain conditions, in sea water, which eliminates a salt effect.

It is probable, however, that the heavy isotope of hydrogen influences the polymerization, since Bingham and Stevens[28] find that concentrated heavy water is inert due to low association (low trihydrol content) and, as Frerichs[29] remarks, workers on the biological effect of concentrated heavy water have not

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[11] Bancroft and Gould, *Jour. Phys. Chem.* 38: 197, 1934.

[12] Wills and Boeker, *Phys. Rev.*, 42: 687, 1932.

[13] Boeker, *Phys. Rev.*, 43: 756, 1933.

[14] Cabrera and Fahlenbach, *Zeit. Phys.*, 82: 759, 1933.

[15] Baker, *Jour. Chem. Soc.*, 130: 949, 1927.

[16] Menzies, *Proc. Nat. Acad. Sci.*, 18: 567, 1932.

[17] La Mer and Miller, *Phys. Rev.*, 43: 207, 1933.

[18] H. T. Barnes, "Ice Engineering," Renouf, Montreal, 1928.

[19] Menzies, *Jour. Am. Chem. Soc.*, 43: 851, 1921 .

[20] Maass and Mennie, *Proc. Roy. Soc.*, 110A: 198, 1926.

[21] Hill, *Proc. Roy. Soc.*, 106B: 477, 1930.

[22] Gortner and Gortner, *Jour. Gen. Physiol.*, 17: 327, 1934.

[23] W. H. Barnes, *Proc. Roy. Soc.*, 125A: 670, 1929.

[24] Plyler, *Jour. Optical Soc. Amer.*, 9: 545, 1924.

[25] Altberg and Troschin, *Naturwissenschaften*, 19: 162, 1931.

[26] Collins, *Phys. Rev.*, 26: 771, 1925.

[27] Kolkmeijer and Favejee, *Nature*, 132: 602, 1933.

[28] Bingham and Stevens, *Jour. Chem. Phys.*, 2: 107, 1934.

[29] Frerichs, *Naturwissenschaften*, 22: 113, 1934.

considered the polymerization factor. Uhlmann[30] has suggested that an isotopic fractionation occurs in the sublimation of ice and snow, which would mean that our aged ice samples contained more deuterium than the condensed water; and the first biological experiments[31] with heavy water showed that a concentration slightly higher than that in "ordinary" water (if there is such a substance) has a beneficial effect on *Spirogyra.*

The term "trihydrol" has been used for the highest polymer, but this may be an aggregate of as many as twenty-three molecules,[32] hexahydrol,[33] a quartz-like structure,[34] or a doublet of the pyramidal anion $H_3O_2$ with the hydrogen ion.[35] Since the polymers differ in density (Sutherland calculates the density of trihydrol as 0.88) separation might be effected by other methods. Berkeley[36] has suggested centrifugal force for the separation of isotopes and polymers, and perhaps ultracentrifuges like those of Svedberg[37] or Beams and Pickels[38] will ultimately develop fields of sufficient magnitude.

The conclusion appears to be that, although ice water and steam water have different biological effects, a good deal more information is needed on the physical side before definite rôles can be assigned to the many forms of water in living matter.

T. CUNLIFFE BARNES
OSBORN ZOOLOGICAL LABORATORY
YALE UNIVERSITY

## A POSSIBLE EXPLANATION OF THE FUNCTION OF GLUTATHIONE IN DEVELOPMENTAL GROWTH

FOR the past two and a half years I have been studying the developmental reaction of *Obelia geniculata* to the naturally occurring tissue "Bausteine." Since some time must elapse before the data as a whole will be published, I am reporting the essence of the results with the three amino-acids of which glutathione is composed. These results derive from observation of the development of some thousands of animals under conditions described in previous papers. As has been told in many reports, the chief function of cystine or cysteine, its reduced form, in developmental growth is acceleration of cell multiplication. This derives from the SH group potentially or actually contained therein. Glycine, a second of the amino-acids of glutathione, has now been found to favor the regeneration of new hydranths from broken pedicels. No other amino-acid does this specifically. The finding suggests that glycine is concerned in the protein reconstitution essential for regeneration. It is perhaps a scientific demonstration of why gelatin with its high glycine content has been popularly supposed to be particularly useful as an article of diet in convalescence from wasting disease. It is consistent with the recent reports in medical literature that in some cases glycine is apparently of benefit in rebuilding muscle tissue. The third amino-acid of glutathione is glutamic acid. And this decidedly and definitely favors the process of differentiation and consequent organization. No other amino-acid yields like effect to like degree. It is this amino-acid which shows as its outstanding and specific influence upon developmental growth the acceleration of differentiation and consequent organization.

Thus, then, it seems as if in glutathione nature has developed in one and the same chemical compound a complex which conditions if it does not determine the course of the several basic and essential processes concerned in developmental growth. Through cysteine it accelerates cell proliferation, the first step; through glycine it accelerates that protein reconstitution which is an essential accompaniment to both cell division and cellular differentiation; and through glutamic acid it accelerates the progress of that selective building-up of the protein molecule which is the characterizing process of differentiation and its consequent organization.

There are those who deprecatingly insinuate that reports of what happens under certain conditions are of little value without explanations of the mechanism producing the given reaction. It takes but little clear thinking to realize that the first step in scientific inquiry is to find out what happens. Only when this has been done can there be found out how it happens. The why is so frequently a subject of metaphysics that it need not be discussed here.

FREDERICK S. HAMMETT
MARINE EXPERIMENTAL STATION
NORTH TRURO, MASS.

# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## A METHOD FOR DESTROYING INTERNAL CELL MASSES

THE problem of destroying internal cell masses with a minimal destruction of the intervening layers of tissues has arisen in a number of different fields of research. Most of the techniques thus far devised have certain limitations which obviate their use in investigations where a precise control over the amount

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[30] Uhlmann, *Naturwissenschaften,* 22: 119, 1934.
[31] Barnes, *Jour. Am. Chem. Soc.,* 55: 4332, 1933.
[32] Duclaux, *Compt. rend. Acad. Sci.* 152: 1387, 1911.
[33] Pennycuick, *Jour. Phys. Chem.,* 32: 1681, 1928.
[34] Bernal and Fowler, *Jour. Chem. Phys.,* 1: 515, 1933.
[35] Kinsey and Sponsler, *Proc. Phys. Soc.,* 45: 768, 1933.
[36] Berkeley, *Nature,* 120: 840, 1927.
[37] Svedberg, SCIENCE, 79: 327, 1934.
[38] Beams and Pickels, *Jour. Chem. Phys.,* 2: 143, 1934.