RECENT ADVANCES IN THE STUDY OF THE BIOLOGICAL EFFECTS OF HEAVY WATER[1]

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In a previous communication the writer (1934a) presented a survey of the work done in an effort to determine the biological or physiological effects of the heavy isotope of hydrogen on cells and tissues. Some mention was made of this new aspect of the physiology of water by Gortner (1934). Bonhoeffer (1934) and Brandt (1934) reviewed the early work. The most complete summary of the literature is that of Barnes and Jahn (1934). It seems proper to consider the more recent work in the field in view of certain previously advanced hypotheses concerning the role of heavy hydrogen in physiological processes with particular emphasis upon that work which points toward an important use of the isotope.

Further work by Richards (1934) indicates that water containing 1:2000 D increased the growth of yeast by 11 per cent compared to distilled water. The cells in the heavy water were more uniform and the total volume of the cells was greater. The deuterium brought about an earlier reorganization within the cells, an action which normally occurs as a culture medium becomes less favorable. On the other hand, the heavy isotope stimulated the growth of the cells for there was a considerable increase in the dry weight of cells grown in the isotope medium.

Recent experiments of Bonhoeffer (1934) indicate that yeast and *Bombardia tetaspora* are not killed by heavy water but their development is retarded.

Anderson and Harvey (1934) found that a solution containing 81 per cent heavy water increased the total amount of light emitted from a certain quantity of luciferin by an average of 19 per cent. The rate of the reaction at half completion averaged about 60 per cent of the control value in distilled water.

Taylor and Harvey (1934) observed the effect of water containing deuterium oxide on the respiration of yeast. No inhibitory effect was noted in concentrations of less than 20 per cent. A solution of 45 per cent D_2O caused a 5 per cent inhibition, 60 per cent brought about a 13 per cent decrease, 72 per cent slowed the rate of respiration 20 per cent, 86 per cent about a 27 per cent inhibition, and 97-98 per cent heavy water caused an inhibition of 50 per cent after one hour. It was noted that the effect tended to increase after two hours.

Larson and Barnes (1934) found that flatworms kept in dilute heavy water for long periods showed a striking difference in the rate of shrinkage of body size when compared to control organisms kept

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in ordinary water. It was also reported that there was a marked increase in mould growth in 0.47 per cent deuterium oxide. The suggestion was made that the property of deuterium in stimulating the growth of moulds might afford some very interesting problems in parasitology and might even present a possible therapeutic use of heavy water.

Further experiments by Barnes and Larson (1934) support the original suggestion made by Barnes (1933) that low concentrations of D_2O might be expected to yield more significant physiological results than the higher concentrations of that substance. The heavy water used contained one part in 2,000 of deuterium. Filaments of *Spirogyra* sp. lived much longer in the isotope water. Planarians maintained their body size for longer periods in water containing D. The fermentation by a suspension of commercial yeast and the digestion of starch by amylase were inhibited after a period of incubation of the enzyme in heavy water. This suggests that the results obtained with *Spirogyra* sp. and *Planaria* sp. were due to reduced enzyme hydrolysis.

Lockemann and Leunig (1934) observed the effect of water containing from 0.020 per cent to 0.54 per cent D_2O on the growth of *Escherichia coli* and *Pseudomonas aeruginosa* using the development of turbidity as the growth criterion. Heavy water containing less than 0.54 per cent deuterium favored bacterial growth. The effect was more rapid and pronounced on *Escherichia coli* than on *Pseudomonas aeruginosa.* No effect was observed within the five-minute period, the differences occurring after thirty minutes.

The reported stimulative effect of heavy water has led Fox (1934) to make a most interesting conjecture relative to a possible relationship between the concentration of heavy hydrogen in the living organism and the production of cancerous tissue. He points out that, if during a gradual decrease in the total water content of aging animals, some mechanism for a selective retention of heavy water were present, this accumulation might reach a concentration, general or localized, that would be sufficient to stimulate the maximum production of neoplasmic cells just as certain concentrations now seem to stimulate the growth of fungi and algae. It should be observed that no stimulation, or any other effect, has been reported from the investigations that have been carried on with cancerous tissue by Woglum and Weber (1934) and Sugiura and Chesley (1934a and 1934b). The water used in most cases, however, contained deuterium in much higher concentrations than that from which a stimulative effect has been reported. Rea and Yuster (1934) have obtained somewhat different results. While the addition of a 11 per cent D_2O solution to inoculations of rat sarcoma R-39 was without effect, the injection of the solution into and around the tumor every second day for ten days seemed to stimulate growth in 6 of the 50 rats so treated. "However, six (12%) of the tumors injected with heavy water seemed to show a more rapid growth and were slightly larger than any of the controls. The in-

terpretation of this finding is not clear. While it may be due to individual variations in the rats, the rather high incidence (12%) and the fact that such large and rapid growth was not obtained in any of the controls, seems to make this finding more significant." It is possible that the stimulation in this case may have been due to the heavy hydrogen isotope and such an interpretation is in agreement with the results obtained by various workers.

Klar (1934) has questioned the results of all those experiments which indicate that deuterium exerts a stimulative effect. He would attribute the increase in growth which has been noted to the presence of organic impurity rather than to the isotope of hydrogen of mass 2. Klar detected mould growth in "Ohio"-water, isotope water obtained from the Ohio Chemical Company, which was "purified" in the same way as that used in the various investigations reported. He indicated that there was sufficient organic matter present in the so-called "pure" water to support the growth of mould.

Barnes (1934) agreed with Klar that a paraffin compound will support the growth of mould in the absence of other organic materials, a fact which has been known for the last twenty-five years, but drew sharp issue with him relative to the adequacy of the methods used in the purification of the heavy water. Not only that, but he pointed out that in the work of Larson with yeast and Meyer with *Aspergillus* sp. there was already an abundant source of available carbon present in the culture media.

Experiments have been reported by Meyer (1934b) to test Klar's objections. Two types of media were prepared. In one type were placed all of the salts used in Pfeffer's three-salt solution with sucrose added as the carbon source. The other solution contained the same salts but no source of carbon. The solvent in each case was triple-distilled deuterium oxide of approximately 0.5 per cent molecular concentration obtained from the Ohio Chemical Company. Twenty-five cc. of the solution was placed in each of eight Erlenmeyer flasks, four flasks with sucrose in the medium; four flasks without. All of the solutions were freely exposed in the laboratory. In less than a week the salt solution to which sucrose had been added showed a heavy fungus growth; after a period of 44 days no growth of any kind was noted on or in the solutions without a carbon source. If the organic impurity suggested by Klar were present at all, it was neither of a kind nor in sufficient quantity to support the growth of mould. It seems exceedingly unlikely that such an organic compound, if present in a medium already rich in available organic material, would have exerted so pronounced a stimulative effect as that attributed to it by Klar.

The value of heavy water as an indicator was first shown by Hevesy and Hofer (1934a and 1934b). By means of direct determinations, it was observed that an exchange between the H_2O of the body of fish and the D_2O of the medium when fish are placed in 0.5 per cent heavy water took place. The velocity of exchange is so great that

equilibrium is reached in a few hours. A reversal of the process occurs when the fish are transferred from heavy water back to H_2O. Continuing their work in connection with this most interesting phase of a possible use of D_2O, Hevesy and Hofer (1934c) analyzed the urine and water of transpiration of one of the experimenters who had drunk water containing deuterium oxide. By this method it was found that a little of the heavy water appeared in the urine within half an hour, but that the bulk of the D_2O leaves the body slowly, about half being eliminated after nine days. This rate of elimination shows that water which is drunk becomes completely mixed with the water of the body. Since it was observed by Stewart and Holcomb (1934) that the isotope concentration of urine does not differ from that of ordinary water, the use of heavy water as an indicator of certain body processes may become exceedingly valuable. The work of Hevesy and Hofer has been corroborated by McDougal, Verzár, Erlenmeyer, and Gaertner (1934). Water injected into rats, with 1.66 per cent heavy water as indicator, distributed itself throughout the entire body in one hour.

No adequate explanation of the psysiological role of deuterium has yet been presented. The investigations indicate that there is a marked difference in its effect on life processes according to the concentration of the isotope. It is at least reasonable to expect that the effect will be more significant in those deuterium concentrations which more nearly approach the concentrations found in nature. If it is accepted that heavy hydrogen in small quantities does have a stimulative effect and that the high concentrations inhibit physiological processes, the basis will have been laid for a careful examination of the physiological processes of organisms in an effort to determine the exact relationship which the double weight hydrogen atom bears to those processes.

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