Marked fluctions in rate occur independent of temperature, (Nelson,[3] Hopkins[2]). Flow is increased during active shell secretion and by addition of fresh oyster sperm to incurrent water of the male oyster. Sperm is effective in less than one minute, causing ostia to enlarge, permitting more rapid flow. A typical increase is from average flow of 5.9 liters per hour to 11. Initial rise in rate is followed quickly by marked decrease, succeeded by secondary rise to new high level, the duration of which depends on amount of sperm introduced. No response occurs in female oyster unless induced to spawn, in which case flow is markedly reduced owing to reversal of water passage through the ostia associated with expulsion of the eggs, Elsey[4], Galtsoff (unpublished observations).

Since spawning in the oyster is a group response, (Nelson,[5] Galtsoff[1]), increased rate of water flow in the males permits wider and more rapid dissemination of sperm. With its power to stimulate spawning in both females and males, (Galtsoff[1]), the group reaction is more definitely assured, especially where the animals are scattered. Reduced filtration by females during spawning greatly decreases capture of eggs by gills of the parent.

8553 P

Effect of Deuterium Oxide on Action of Some Enzymes.

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Since the discovery of heavy water much speculation of very diverse character has been advanced in regard to its possible effects on life. The most extreme views are presented by those who claim, without adequate experimental proof, that deuterium oxide is a most powerful destructive physiological agent and those who, basing their conclusions on experiments made with deuterium oxide in dilute concentrations, state that its pharmacological effects are of negligible character.[1, 2, 3] The authors have recently dis-

5 Elsey, C. R., *Trans. Roy. Soc. Can.*, 1935, Sec. V, 131, 142.

1 Meyer, *J. Tenn. Acad. Science*, 1934, **9**, 225.

2 Meyer, *J. Tenn. Acad. Science*, 1935, **10**, 111.

3 Macht and Davis, *J. Am. Chem. Soc.*, 1934, **56**, 246.

covered an interesting and striking contrast in the biophysical and biochemical behavior of H_2O and D_2O, which consists of a difference in velocity of reaction shown by certain animal and vegetable enzymes suspended therein. Studies were made on the behavior of muscle oxydase from the rat, of oxidative enzymes of fresh, blood-free brain tissue from the cat, and of reductase of finely ground seeds of *Lupinus albus* by the Thunberg method of determining the rapidity with which a standard buffered solution of methylene blue is decolorized in special vacuum tubes. Over 50 experiments were performed. The method employed is described in full elsewhere.[4, 5] A marked difference was found to exist between identical enzymes suspended in H_2O and in the same kind of water to which small quantities of deuterium oxide had been added. This difference was demonstrated not only with concentrations of D_2O, 1:100, but also with concentrations of 1:2000 and less.

Further studies were made in connection with the influence of deuterium oxide on the activity of another enzyme, catalase. Catalase from fresh rat muscle and from *Lupinus albus* seeds in distilled water and in water to which small quantities of deuterium oxide were added also revealed a difference in the speed of evolution of oxygen gas from hydrogen peroxide in the presence of these enzymes. The method employed was a modification of that described by Baldwin.[6] Briefly, it consisted of the use of a Thunberg tube into which were introduced one fifth molar of disodium phosphate and one fifth molar of diacid potassium phosphate. When the experiment is begun, the catalase suspension is allowed to run into the Thunberg tube and the oxygen gas evolved is collected by

TABLE I.
Experiments with Muscle Oxydase.
Technique: 2 cc. of muscle suspension plus 1 cc. of Thunberg solution introduced into tubes on water bath at 38° C.

Oxydase Prepared with H_2O Saline.		Oxydase Prepared with D_2O Saline, 1:100	
Tube	Decolorization Time	Tube	Decolorization Time
	min.		min.
1	180	1	149
2	186	2	120
3	182	3	115
4	180	4	140
Aver.	182	Aver.	131

[4] Macht and Bryan, *J. Biol. Chem.*, 1935, **110**, 101.

[5] Macht, *Arch. internat. de pharmacodyn. et de therap.*, 1934, **49**, 175.

[6] Baldwin, *Am. J. Bot.*, 1935, **22**, 635.

displacement of water in a fermentation tube connected with the side arm of the apparatus. Tables I, II, and III show the character of the findings obtained in the numerous experiments performed.

TABLE II.
Experiments with Brain Tissue Extract.

Tube	Oxydase Prepared with H_2O Saline (NaCl, 0.9%) — Decolorized in						Tube	Oxydase Prepared with D_2O Saline, 1:2000 — Decolorized in			
	hr.	%	hr.	%	hr.	%		hr.	%	hr.	%
1	1	45	4	75	5½	100	1	1	55	4	100
2	1	45	4	75	5	100	2	1	55	4	100
3	1	45	4	75	5½	100	3	1	55	4¼	100

TABLE III.
Experiments with Catalase of *Lupinus albus*.
Technique: Placed in tube 1 cc. of H_2O, 3 cc. of H_2O_2 (3%), 1 cc. of KH_2PO_4 (M/5), and 1 cc. of Na_2HPO_4 (M/5).

Series 1 (2 experiments) 0.5 cc. bean extract made with H_2O		Series 2 (2 experiments) 0.5 cc. bean extract made with D_2O, 1:100	
Aver. Oxygen 15 min.	3.3 cc.		5.55 cc.

It will be noted that the enzymatic activity was accelerated in the presence of deuterium. In some of the experiments the enzymes exhibited a diphasic activity. This was especially true of more concentrated solutions of D_2O. There was a primary retardation, which was followed by a secondary and more marked acceleration. In all the experiments, however, a definite difference in reaction was noted between H_2O and D_2O media.

These findings have not only a purely theoretical scientific interest but also a practical bearing. It is well known that minute quantities of powerful drugs or poisons are often more readily detected by physiological tests on living animal or plant preparations *in vitro* than by chemical or even physical means.[7, 8, 9, 10] When studying such physiological reactions it is necessary to employ aqueous solutions of definite composition. Minute solutions of organic or inorganic impurities present in water used for physiological tests may profoundly influence the biological reactions. The writers' work indicates that even in case of pure water differences may occur in the physiological, pharmacological or biochemical responses due to variations in the quantity of the hydrogen isotope,

[7] Hunt, *Science*, 1930, **72**, 526.

[8] Macht and Anderson, *J. Am. Chem. Soc.*, 1927, **49**, 2017.

[9] Hatcher, *American Druggist*, 1929, **79**, 22.

[10] Macht, *Am. J. Ophthalmol.*, 1931, **14**, 726.

deuterium, present in the sample used. When we recall that there are 2 known isotopes of hydrogen—namely, deuterium and tritium —and that 3 varieties of oxygen, differing slightly in their atomic weight, have been established, the subject appears to be even more complex than we at first suspected.*

8554 C

Assays of Hypertrophied Prostatic Tissue and of Urine for Estrogenic Substances.

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A high content of oestrin has been reported in normal ovarian tissues[1] and in tumors of the ovary,[2] breast,[3] and uterus.[4] Estrogenic substances have been demonstrated in the blood and urine, and in some cases a fairly high yield has been reported from male urine.

High yields of anterior-pituitary-like hormone have been obtained from urine in cases of teratoma of the testis,[5] chorio-epithelioma and hydatidiform mole,[5, 6] but Smith and Smith[7] have reported low levels of oestrin in such cases.

There have also been reports of atypical growth of prostatic

*Since this paper was written there has appeared in these PROCEEDINGS (1936, **33**, 266) an article by Fox and Craig concerning the effect of deuterium on the hydrolysis of starch. This biological reaction was found to correspond qualitatively with those described in the foregoing paper.

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1 Allen, E., Pratt, J. P., and Doisy, E. A., *J. A. M. A.*, 1925, **85**, 399.

2 Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1204.

3 Lewis, D., and Geschickter, C. F., *J. A. M. A.*, 1934, **103**, 1212.

4 Lewis, D., and Geschickter, C. F., *J. A. M. A.*, 1935, **104**, 45.

5 Ferguson, R. S., *Am. J. Cancer*, 1933, **18**, 269.

6 Geschickter, C. F., Lewis, D., and Hartman, C. G., *Am. J. Cancer*, 1934, **21**, 828.

7 Smith, G. van S., and Smith, O. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 847.