

simultaneous creatinine and organic iodine clearances; the latter for convenience are expressed in terms of iodine alone. Points on the horizontal line, intercepting the ordinate at 1.0, indicate that the clearances of organic iodine and creatinine were equal. The distance by which a given point is above or below that line shows the amount by which the iodine clearance exceeded or fell short of the simultaneous creatinine clearance, *i. e.*, the magnitude of the ratio, $\frac{\text{cc. plasma cleared of iodine per minute}}{\text{cc. plasma cleared of creatinine per minute}}$. This ratio is charted against the average concentration of iodine in plasma during the clearance period.

Skiodan clearances were approximately equal to creatinine clearances over a wide range of plasma Skiodan concentration. Neoskiodan and Hippuran clearances, however, were of the same order of magnitude as the creatinine clearances only when the concentration of Neoskiodan, or Hippuran, in plasma was high. They became several times greater than creatinine clearances when the concentration of Neoskiodan or Hippuran in plasma approached zero.

It appears, therefore, that the mammalian (dog and man) kidney excretes Skiodan and creatinine at approximately the same rate relative to plasma level. Under certain conditions Neoskiodan and Hippuran clearances have the same order of magnitude as the simultaneous creatinine clearances. The mammalian kidney can, however, concentrate Neoskiodan and Hippuran more highly than creatinine when their respective concentrations in plasma approach zero. This relationship provides a new tool for investigating renal function. The mechanism by which the kidney excretes these substances is being studied further.

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Effects of Deuterium Oxide on Respiration of Germinating Seeds.

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Lewis,¹ the first to report on the biological effects of heavy water, states that tobacco seeds do not germinate in nearly pure deuterium oxide, and that they do so very slowly in water containing 50%

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¹ Lewis, G. N., *J. Am. Chem. Soc.*, 1933, **55**, 3503.

deuterium oxide. In view of the new field of research which this finding would open up, if confirmed, it seemed desirable to obtain a quantitative measurement of the effect of deuterium on germination. For this reason we have made a study of the rate of aerobic respiration of wheat seeds in the early stages of germination.

It is necessary to determine first, whether deuterium oxide inhibits the germination of seeds. If so, does it act as a toxic agent, or if mixed with ordinary water, does it simply dilute the ordinary water until the latter is present in insufficient quantity to support germination? If the deuterium oxide acts as a toxic agent, is its action evident before or after the beginning of karyokinesis?

The respiration of wheat seeds under different conditions was followed by means of a Warburg respirometer. To bring about germination with very small volumes of water, most of the endosperm portion of the seed, whose rate of respiration is low, was discarded, and the experiments were performed with the end of the seed containing the embryo and a small portion of reserve material, it having been found by experiment that this procedure did not interfere with germination in ordinary water.

For each experiment the embryo ends of 10 seeds were placed in the central portion of each of 4 small respirometer (Warburg) vessels. Five hundredths cc. of heavy water were then added to the seeds in each of the 2 experimental vessels and 0.05 cc. of ordinary distilled water to the seeds in each of the control vessels. The seeds were allowed to germinate for different lengths of time at 26°C. Two-tenths cc. of 7% NaOH were then placed in the outer portion of each vessel and the rate of respiration at 26°C. was determined over periods. The conditions of light and O₂ supply were the same for experimental and control series. The experimental error of the method was such that differences between experimental and control figures of less than 10% can not be considered significant.

Since the quantity of heavy water available was small, it was necessary to determine the minimum volume of water necessary to

TABLE I.
Respiration of 20 Embryos in Different Quantities of Water 21 Hours after Beginning of Germination.

Wt. dry embryos mg.	Period of obser- vation, Min.	Vol. O ₂ consumed, mm. ³ Seeds in 0.1 cc. H ₂ O	Seeds in 0.15 cc.
102	15	31.5	38.5
	30	64.8	79.4
80	15	31.5	35.0
	30	59.7	68.0
	45	96.4	103.6
	60	118.3	131.8

support satisfactory germination in a given number of seeds. From the figures given below it is evident that 0.1 cc. of ordinary water will cause germination in 20 embryos, but that the rate of respiration is significantly higher with 0.15 cc. than with 0.1 cc.

As it was difficult to know with accuracy the pH of each sample of heavy water used (since the behavior of the deuterium ion towards indicators is not yet well known), it became necessary to test how far the respiratory rate would depend on changes in the pH due to an impurity as traces of NaOH.

TABLE II.
Respiration of 20 Embryos in Solutions of Different pH.

Wt. dry embryos	Age	Period of Observation	Vol. O ₂ consumed.	Vol. O ₂ consumed.
			Seeds in 0.1 cc. ordinary distilled water pH 5.5	Seeds in 0.0003 N NaOH pH 9.0
mg.	hr.	min.		
78	21½	15	19.7	20.4
		30	55.8	56.5
	34	15	37.3	41.5
		30	76.4	87.7
80	18½	15	22.8	23.4
		30	66.4	65.8
	43	15	35.1	49.6
		30	78.6	107.2

No differences in the respiratory rates of the seeds in the 2 solutions were observed on the first day, but differences began to be apparent on the second day.

Preliminary work was done with 14.8% *deuterium oxide*. A series of 20 embryos were allowed to germinate for 13 hours in 0.1 cc. of ordinary water, another series of 20 embryos in heavy water, and the respiratory rate measured. At this time imbibition was active, but karyokinesis had not begun. An additional 0.1 cc.

TABLE III.
Respiration of 20 Embryos in 14.8% Deuterium Oxide.

Wt. dry embryos	Age	Period of Observation	Vol. O ₂ consumed, mm. ³ .	Seeds in 0.1 cc. ordinary distilled water	
			Seeds in 0.1 cc. 14.8 % deut. ox.	distilled water	
mg. 120	hr. 13	15	34.3	36.5	
		30	66.5	67.8	
		45	97.6	101.4	
		60	128.2	136.6	
		14	0.1 cc. 14.8 deut. ox. and ordinary distilled water added at this time.		
			15	35.3	35.2
	40	30	74.6	74.6	
		45	113.0	112.0	
		60	157.6	156.7	

of water was then placed on the seeds, they were allowed to germinate for another 24 hours, and the respiratory rate was measured again. At this time karyokinesis and visible growth had begun. The results are given in Table III.

No significant difference was observed in the respiratory rates of the seeds in ordinary and heavy water. It is evident from this experiment that deuterium oxide has no pronounced toxic action on the germination of wheat seeds, before or after karyokinesis sets in.

The effect of 38% deuterium oxide was then studied.

TABLE IV.
Respiration of 20 Embryos in 38% Deuterium Oxide.

Wt. dry embryos	Age	Period of Observation	Vol. O ₂	Vol. O ₂
			consumed mm. ³ Seeds in 0.1 cc. 38% deut. oxide	consumed mm. ³ Seeds in 0.1 cc. ordinary distilled water
mg.	hr.	min.		
82	16.30	15	29.0	28.6
		30	55.8	59.3
		45	93.5	94.1
		60	120.9	121.8

Table V shows the results of experiments with 94% deuterium oxide. This material had the same pH as ordinary distilled water when measured under the same conditions.

The question arises whether sufficient ordinary water might have distilled from the NaOH solution into the 94% deuterium oxide to cause germination in the seeds. This is unlikely, since the NaOH remained in the Warburg vessels only during the time when respiration measurements were being made, and since the volume (0.2 cc.) of NaOH solution used was small. In order to show more definitely that the germination of the seeds in 94% deuterium oxide was not due to such absorption, dry embryos were placed in the inner portion of the Warburg vessel and 0.5 cc. NaOH in the outer. The seeds did not take up sufficient water to germinate although observed for several days. Furthermore, it was found that seeds germinated readily in 94% deuterium oxide in a closed vessel containing no NaOH solution.

It is evident that, except for the reading made during the first hour of germination when the percentage experimental error is high, there is no significant difference in the respiratory rates of embryos in ordinary and heavy water during the first 2 days of observation. However, on the second day karyokinesis was active. Since 0.1 cc. of 94% heavy water contains only 0.06 cc. of ordinary water, and since we have shown above that the respiratory rate of

TABLE V.
Respiration of 20 Embryos in 94% Deuterium Oxide.

Wt. of dry embryos	Hr. germination	Time of observation	mm ³ O ₂ consumed					
			Exp. 1		Exp. 2		Exp. 3	
			94% A	Dist. B	94% A	Dist. B	94% A	Dist. B
mg. 80	0*	1st ½	6.9	11.0	2.1	4.1	5.2	8.2
		2nd ½	10.0	8.1	4.3	8.2	7.1	7.8
		2nd	18.5	17.9	17.4	20.5	17.6	20.9
		3rd	22.3	25.6	21.5	22.1	23.0	24.7
		4th	26.0	26.8	24.1	26.7	24.2	26.9
		5th			27.5	26.5	27.0	27.2
		6th					30.6	31.0
	24†	1st ¼	29.4	29.8	{ }	{ }	31.9	35.9
		2nd ¼	29.2	29.8	{ 57.5 }	{ 59.2 }	29.8	27.5
		3rd ¼	34.6	31.5	{ }	{ }	{ }	{ }
		4th ¼	30.4	28.3	{ 59.0 }	{ 57.5 }	{ 56.4 }	{ 54.8 }
	25*	1st ¼	30.3	36.2	{ }	{ }	{ }	{ }
		2nd ¼	30.7	38.5	{ 67.7 }	{ 80.3 }	{ 70.8 }	{ 75.2 }
		2nd ½			74.7	88.9	79.6	92.0
		3rd ½			78.3	91.8	111.8	139.2
		4th ½			83.6	97.1	164.6	185.2
		5th ½			91.5	106.7		
	48	1st ¼	39.4	40.6			23.5	24.1
		2nd ¼	38.4	38.2			22.9	24.8
		3rd ¼	39.1	42.3			25.5	28.4
		4th ¼	42.5	44.3			23.8	27.3

*0.1 cc. of 94% heavy water and of ordinary distilled water added to the organisms in the experimental (A) and control (B) vessels, respectively. Measurements taken as soon as the apparatus was again in temperature equilibrium.

†Measurements made before any new addition of water.

20 embryos in ordinary water is sensitive to changes in the volume of water available in the range 0.1-0.15 cc., it appears that the heavy water does not act simply as a diluent to ordinary water, but that it enters into the life processes in a manner similar to ordinary water.

Summary. A study has been made of the germination of wheat seeds (*Triticum vulgare*) in 14.8%, 38% and 94% deuterium oxide, and ordinary water for control, under as nearly identical conditions as possible. In all cases the seeds germinated and no macroscopical differences could be detected. No significant differences were observed in the respiratory rates of the seeds in ordinary and heavy water during the first and second days of germination.

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