THE RESPIRATION OF BARLEY DURING GERMINATION, USING THE CONDUCTIVITY METHOD FOR THE ESTIMATION OF CARBON DIOXIDE.

By

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I. INTRODUCTION

Because of its steadily increasing economic importance, barley, along with other cereal grains, has been the subject of a considerable volume of research in the last decade. At the present time the annual barley production in Canada exceeds one hundred million bushels, with an estimated value of thirty-three million dollars, making it the third largest cereal crop in this country. Over half of the annual crop is used for feed, with the remainder being used for export, seed, and brewing, although export has been at a minimum since 1939, due to limited shipping facilities. In connection with both storage and brewing the problems arising are largely physiological and consequently an attempt has been made in this research to obtain accurate data on the water absorption and respiration of barley, with a view to understanding the complicated processes involved in germination of the seed. Up to the present time the majority of the research work on barley has dealt with analysis of the enzymes and their products during malting and respiratory data deal only with the fully developed seedling. No data are available on the early period of germination, yet it is during this period of activation that very significant changes in the enzymes and substrate take place. The first section of this thesis deals with the water absorption of barley

under various experimental conditions, while the latter part deals with the respiration during the first 24 hours of germination.

In dealing with respiration, there are several methods of attacking the problem, each with its own merits and demerits. For example, in the Warburg manometric method the oxygen consumption is estimated and taken as an index of the respiratory intensity. The disadvantage of this system is obvious when the respiration of certain substrates such as fats is considered. In this case the breakdown to simple sugars is accompanied by the release of oxygen and no indication of this source is shown by such a method which only estimates the atmospheric oxygen consumed, giving an inaccurate picture of the intensity of respiration. Another method which presents itself is that of progressive estimation of the substrate being respired. While such a method gives interesting data on the changes in respirable material, the complicated and tedious process of quantitative analysis of the plant material preclude the possibility of such a method from general use. The most generally used and most satisfactory method of measuring the respiratory intensity is that of CO_2 estimation. If sufficiently large quantities of the gas are being produced, samples may be removed from the respiration chamber at intervals and the changes in composition of the gas mixture may

be determined by the gas analysis method. Refined modifications of this system have been developed to analyse very small amounts of gas, but the principle remains the same. By passing the air stream with the CO_2 through certain indicator dyes, color changes are produced and by comparison with standard samples of the dye, the carbon dioxide content of the mixture may be determined. Another method is the absorption by soda-lime and weighing before and after absorption. The CO_2 may be absorbed in standard solutions of barium hydroxide and the CO_2 content after absorption determined by titration. The carbon dioxide can similarly be absorbed in other alkaline solutions such as sodium or lithium hydroxide and titrated. The katharometer, as developed by Stiles and Leach,(1), is by far the most sensitive and most accurate means of measuring the CO_2 output of small quantities of material. While other systems require relatively large amounts of respiring material to give significant results, the katharometer has the advantage of giving a continuous record of the respiration of one seed. Consequently, changes in the rate of respiration of the individual seed are not masked by variations in the development of each seed, as occurs when a group of seeds are used. To replace the rather tedious method of absorbing CO_2 and titrating against acid solution, Spoehr (2), has applied the conductivity method of CO_2 estimation to the

problems of plant physiology. The use of this method depends on the fact that the electrical conductivity of the absorbing solution decreases as the carbon dioxide is absorbed, and the magnitude of the change in the conductivity is a measure of the carbon dioxide. The principle of this method has been applied in the present work, and a complete description of the apparatus used and the problems involved are described in detail in a later section.

Two varieties of barley were used throughout the present work and the writer is indebted to the kindness of Mr. W. O. S. Meredith of the Dominion Malting Laboratory, for providing the material. The variety O.A.C.21, the standard Canadian malting barley was used for the majority of the experiments, but additional tests were carried out on another variety, Wisc. 38, with a view to exhibiting varietal differences. These two varieties were chosen because of their marked difference in malting properties.

Before proceeding with a detailed description of the methods and results obtained on water absorption and respiration, some general information on the grain of barley may be of interest. Barley (Hordeum sativum Jensen) belongs to the same tribe as wheat and rye and differs from both in that the spikelets are one-flowered and in having more than one spikelet at the joint of each rachis. There are two well-marked types of

barley.

(1) six-rowed barley (Hordeum sativum hexastichon Haechel)

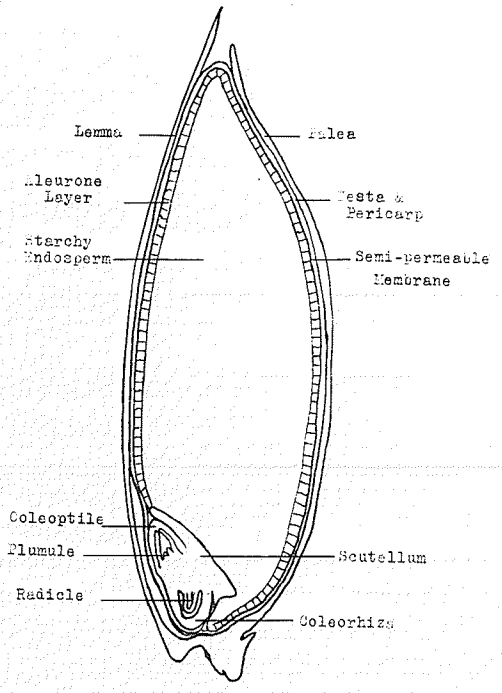
(2) two-rowed barley (Hordeum sativum distichon Haechel)

In the six-rowed type there are three florets each bearing a single grain arranged alternately at each joint of the rachis, thus making a spike with six rows of grains. If the lateral grains of the alternate sets overlap, they form one row in place of two and give the appearance of a four-rowed barley. In the two-rowed type the lateral grains have failed to develop through the abortion of the ovary. The joints of the rachis are further apart in this type and the grains are naturally fewer in number. There is a rather uncommon form which is a true four-rowed barley, due to the abortion of the ovule of the middle floret. Except in the rather rare hull-less varieties, the barley kernel remains enclosed in part of the leaf system of the plant, which is termed the hull, or husk. It consists on the adaxial side of the palea superior and on the abaxial side of the lemma, or palea inferior, which is usually prolonged into an awn of varying length, being absent in some varieties. The lemma slightly overlaps the palea and is the more readily removed of the two. It is the lemma that forms a protective sheathe over the embryo of the grain. In the varieties investigated in this work the hull made up about 10% of the total weight. When the hull is removed the kernel that remains

is the caryopsis, which is strictly speaking a fruit and is very similar in appearance to a wheat grain. The hull is attached to the kernel by the secretion of a sticky substance during development and this combination of hull and kernel is referred to as the grain.

The barley kernel is made up of the embryo and endosperm. The embryo occupies about one thirtieth of the total weight of the grain and is the seat of great respiratory activity during germination. The plumule is sheathed by a coleoptile, while the radicle is capped by a coleorhiza. Attached to these organs and closely pressed against the endosperm on the other side is the scutellum. This organ is responsible for absorbing nourishment for the embryo, which it does by the secretion of enzymes into the endosperm, causing the mobilization of food reserves. The endosperm consists largely of thin-walled isodiametric cells, filled with starch grains. Surrounding the endosperm and terminating at the scutellum is the aleurone layer, two or three cells in the thickness, containing protein and fat. The structures described may be readily seen in the accompanying diagram. (Fig. 1.).

As indicated by the table, carbohydrates and carbohydrate derivatives constitute approximately 75% of the total content of the grain. In the samples used the original water content was close to 11%, and was retained at that value throughout the experiments by storing in



L. S. of Barley Grain.

After Kind.

Fig. 1

air-tight containers. It will be shown later that barley will readily take up water from a moist atmosphere.

The following table, from Hind, H. L. Brewing, Vol. 1, gives a generalized analysis of barleys;

	Two-Rowed	Husky Six-rowed
	%	%
Starch	60	52
Sugars	2.5	2.5
Hemi-celluloses, Pectins, etc.	8	11
Cellulose	4	7
Lignin	1.5	3
Protein and other nit. substances.	9	9
Fat (ether extract, contains traces resins)	2.5	2.5
Ash	2.5	3
Tannin	Small quantity	
Water	10	10

Another interesting characteristic of barley is the presence of semi-permeable membrane investing the kernel. This membrane has been the object of a considerable volume of research in the past and its development and significance will be discussed in the section dealing with water absorption.

II THE WATER ABSORPTION OF BARLEY.

During the course of the experimental work on respiration of barley the rate of water absorption of the barleys being used was determined under the various experimental conditions. As indicated in the introduction, the presence of a semi-permeable membrane in barley has led several workers to investigate the absorptive powers of barley. Although the presence of a semi-permeable membrane in the seeds of a large number of plant families was first pointed out by Gola, (4), in 1905, the first quantitative work on water absorption by barley was published two years later by A. J. Brown (5). The latter demonstrated conclusively that the grain of Hordeum is enclosed in a semi-permeable or selective covering which permits the passage of water or iodine to the interior of the grain, but which prevents the passage of sulphuric acid and hydrochloric acid, but not nitric, --the latter due to the destructive action of the acid on the membrane. Certain salts in aqueous solution are also prevented from entry by the membrane. He also showed that the semi-permeable property of the covering of the grain of Hordeum is not due to the action of living protoplasm. Brown also showed that the semi-permeable property of the covering is located in the spermoderm of the grain. The spermoderm is located just outside the aleurone cells of the endosperm and is probably derived from the epidermal layers of the nucellus.

About 10 years later, Pickler (6), published the results of his investigation of the diastatic activity of barley seeds and its relation to their moisture content, using White Hull-less barley. Although Pickler's work deals with water absorption from saturated lithium chloride solution, he also gives data for absorption of distilled water. Wolfe, (7), in 1926, repeated Pickler's work using the same variety of barley and showed strong disagreement with the former's results. A discussion of these results will be given in a later section.

In the present work the water absorption of barley in distilled water only was investigated. Although the majority of the experiments were conducted on the variety O.A.C. 21, the absorption of the normal grain of Wisc. 38 was also determined. All experiments were conducted at a temperature of 25°C.

Experimental procedure:

For the initial determinations the water absorption was determined by the usual method of blotting and weighing the grains after progressive exposure to distilled water. A standard technique for blotting and weighing was used throughout and although this method is somewhat tedious, the results are very satisfactory. The error of the method was determined by weighing the dry grains, immersing in distilled water momentarily to wet the surfaces, blotting and weighing again. Several trials were made in this manner, and an average value

was determined, to be subtracted from the total weight after each interval. This factor compensates for the error introduced by the moisture which cannot be readily removed by blotting. Weighings were made at half hour intervals for the first two hours and at hourly intervals from that point on. To obtain complete data for the 40 hour period over which most of the investigations extended, duplicate samples were used and started 12 hours apart. This method gave very satisfactory results and had an added advantage, in that the smooth curve which invariably resulted showed that the samples checked closely with one another at points where they met. For use with the blotting method, samples of 2 grams (about 50 grains) were used. For the greater part of the work on water absorption, a centrifuge method was used. Two pairs of brass tubes were obtained about 2 cms. in diameter and 6.5 cms. in length. Fine mesh copper screen was firmly soldered across the bottom of each tube, and the opposite end was flared out to catch in the collar of the centrifuge. The centrifuge was a hand-operated model and after some practise the technique was standardized as to the number of turns and speed of operation. One gram samples of barley were placed in the tubes and the error of the method was determined as before, by immersing the tubes and barley in distilled water after weighing, centrifuging immediately, and calculating the difference.

This was added to the weight of the tubes and the total subtracted after each weighing. The foremost advantage of the centrifuge method is that it obviates the necessity of handling the sample of barley at each weighing. Two samples can be centrifuged and weighed in slightly more than two minutes, while the blotting method requires more than twice that time. Another advantage is that the centrifugal force will remove surface water from the furrow of the grain, while it can be removed only with difficulty by blotting. In the case of the water absorption of barley in contact with water which is discussed in a later section, the blotting method was used because the centrifuge method did not lend itself to conditions where the grains must be spread out in a single layer.

The original water content of the barley was determined by drying weighed samples to constancy in a dry air oven at 100°C. The water content of the variety O.A.C.21 was found to be 10.9%, while that of the variety Wisc. 38 was 11.0%. As previously mentioned, the samples were stored in air-tight containers to prevent further changes in water content.

All data on water absorption are expressed as percentage water content of the grain at the time of weighing, i.e. $\frac{\text{Increase in weight}}{\text{Total weight}} \times 100$

To this figure was added the original water content of the fresh seed.

The data for the normal grain of the variety O.A.C.21 are plotted as shown in Fig. 2a. The curve rises steeply for the first few hours and then gradually flattens out as the grain approaches saturation. At 44 hours the water content of the grain is 50% and the curve is still rising slightly. Determinations have been made over periods up to 85 hours in length and the curve still showed a very gentle upward slope although it appeared to be reaching an equilibrium.

In the majority of the water absorption experiments, the grains were completely immersed in water. While this was the most convenient means of exposure to water, the results cannot justly be used to determine the water content of grains being used in respiration experiments as they are not completely submerged in the respiration chamber. Consequently the water absorption of barley grains was determined under respiration chamber conditions in which the grains are placed in a single layer on filter paper with sufficient water to creep up over the surface of the grain only. The results are shown as plotted in Fig. 2a. Under these conditions germination proceeds quite rapidly and determinations cannot be made for the full 48 hour period, as blotting necessarily breaks off some rootlets. Unless otherwise stated, all other absorption curves are for grains completely immersed. The germination under such conditions is retarded possibly due to lack of oxygen. The water absorption proceeds at a slightly higher rate when the grain is in

contact with water, as compared with when it is totally immersed in water.

Another experiment was conducted over a period of 10 days, to determine the rate of water absorption from saturated air. A brass chamber of about 2 litre capacity was almost completely immersed in the constant temperature bath. A screen was secured about an inch from the bottom and below this was placed absorbent cotton with a surplus of water to maintain a saturated atmosphere. A tight fitting glass lid was placed over the chamber. The barley samples were placed on watch glasses on the screen and weighed at intervals. Water absorption proceeded slowly, but the curve as shown in Fig. 2a is of the same general form as the others. Although germination may have commenced eventually, the samples were removed after 10 days because of the growth of Cephalothecium roseum and other fungi on the samples. The water content at this time was 34.1%.

The rate of water absorption of killed grains was also determined. Samples were killed by two methods. One lot of the grain was exposed to chloroform vapor and all grains were killed after 170 hours exposure as indicated by germination tests. The most satisfactory method of killing the grain was by soaking in distilled water for a few hours and then drying at 100°C. to the original weight. This method was much faster and showed a perfect kill when germination tests were tried.

Samples prepared in this manner were placed in the centrifuge tubes and the water absorption was determined in the usual manner. The results were plotted and are shown in Fig. 2a. The curve shows a more rapid initial rise than the curve for normal barley immersed in water and as shown in the diagram is almost completely superposed on the curve for barley in contact with water.

During the course of the later part of the work on respiration, the hulls of samples of barley were partially and completely removed to determine the effect on respiration. Similarly, experiments were conducted to investigate the effect of the hull on water absorption. With the aid of a dissecting lens and a sharp-pointed scalpel, the hulls of several grams of O.A.C. 21 barley were completely removed. As indicated previously, the lemma is quite readily removed, but the palea, on the furrowed side of the grain adheres much more closely. Although great care was exercised in the removal of the latter, the layers underneath it were unavoidably damaged slightly. This was quite evident from the examination of the swollen grain, as the starchy endosperm stood out quite clearly in spots where the pericarp and testa had been damaged, although examination of the dry grain did not reveal the damage. The water absorption was determined by the usual method and the results were plotted as

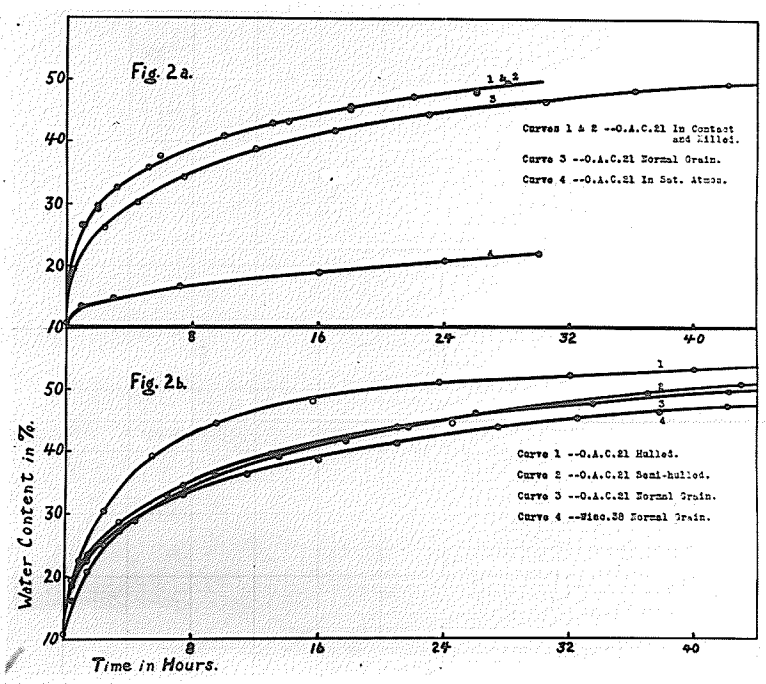


Fig. 2a.
1 & 2
3
4
Curves 1 & 2 --O.A.C.21 In Contact and Milled.
Curve 3 --O.A.C.21 Normal Grain.
Curve 4 --O.A.C.21 In Sat. Atmos.
50
40
30
20
10
8
16
24
32
40
Fig. 2b.
1
2
3
4
Curve 1 --O.A.C.21 Hulled.
Curve 2 --O.A.C.21 Semi-hulled.
Curve 3 --O.A.C.21 Normal Grain.
Curve 4 --Wisc.38 Normal Grain.
Water Content in %.
Time in Hours.

shown in Fig.2b. For purposes of comparison the absorption curve of the normal grain is also shown in Fig.2b. The curve of the completely hulled barley rises more steeply than that of the normal, but also tends to flatten out more rapidly.

The water absorption of O.A.C.21 barley with only the lemma removed was also determined. In this case the lemma could be readily removed with no damage to the tissue lying beneath. It is interesting to note that the curve in this case is almost completely superposed on that of the normal grain, as shown in Fig.2b.

Up to this point all water absorption experiments were conducted with the variety O.A.C.21. As considerable experimental work on respiration was done on the variety Wisc. 38, the rate of water absorption for this variety was also determined. Samples of the normal grain were treated in the usual manner and the results are shown in Fig. 2b. Although the curve is of the same type as that of O.A.C.21, after the first five hours it falls slightly below that of the former and continues at a rate parallel to it for the remainder of the period.

All experiments on water absorption were conducted in duplicate and results invariably agreed to 1%. The weighings were made in most cases at hourly intervals, but only the data necessary to show the trend

of the curve are plotted in Figs. 2a and 2b. The data for the curves are included in the following tables.

TABLE I. Water Absorption Data For Barley (Fig. 2a).

O.A.C.21 Normal		O.A.C.21 in Contact		O.A.C.21 in Sat. Atmos.		O.A.C.21 Killed	
Hour	Water Content	Hour	Water Content	Hour	Water Content	Hour	Water Content
0.	10.9	0.	10.9	0	10.9	0	10.9
.5	19.32	1.0	26.7	1	13.7	1.16	26.5
2.5	25.8	2.0	29.7	3	14.8	2.0	31.6
4.5	30.3	3.25	32.7	7.25	16.7	6.0	37.9
7.5	34.3	5.25	35.8	16.0	18.9	13.0	43.0
12.0	39.1	10.0	40.9	24.0	20.9	18.0	45.0
17.0	41.9	14.0	43.1	30.0	21.7	26.0	47.9
23.0	44.5	18.0	45.6	120.0	28.5		
30.5	46.6	22.0	47.3	222.0	34.1		
36.0	48.4	28.0	49.4				
42.0	49.7						

TABLE II Water Absorption Data for Barley (Fig. 2b).

O.A.C.21 Normal		O.A.C.21 Hulled		O.A.C.21 Semi-hulled		Wisc. 38 Normal	
Hour	Water Content	Hour	Water Content	Hour	Water Content	Hour	Water Content
0	10.9	0	10.9	0	10.9	0	11.0
1.5	23.3	.5	18.6	.5	16.3	.5	18.75
3.5	38.5	1.0	22.4	1.5	20.8	1.5	22.5
7.5	34.3	2.5	30.4	3.5	27.1	2.5	25.0
13.0	36.6	5.5	39.2	6.5	32.9	4.5	28.8
17.0	38.9	9.5	44.7	9.5	36.3	7.5	32.8
21.0	41.1	15.7	47.8	13.5	39.0	11.5	36.4
24.5	44.4	23.7	51.1	17.7	41.6	16.0	38.5
33.5	47.4	32.0	52.2	21.7	43.9	21.0	41.1
42.25	49.7	40.0	53.1	26.0	45.9	27.5	43.8
				37.0	49.3	32.5	45.2
				43.0	50.9	37.6	46.2
						42.25	47.1

III THE RESPIRATION OF BARLEY DURING GERMINATION

Method:

The conductivity method for the estimation of carbon dioxide has been applied in this investigation to determine the rate of respiration of barley under various experimental conditions. A brief discussion of the history and development of this method follows.

Historical

The first application of this method was not for biological purposes. In 1919, Cain and Maxwell (8) introduced the method to determine the carbon content of steel. The carbon dioxide produced by direct combustion of the metal was passed through barium hydroxide solution of known electrical resistance and the resulting change in the conductivity was determined. The increase in resistance is due to the removal of the barium ion by its precipitation as insoluble barium carbonate. The method as developed by Cain and Maxwell was, biologically speaking, of low sensitivity. The amounts of carbon dioxide being absorbed were relatively large and the authors were interested only in total amounts of the gas.

Spoehr and McGee (2) in 1923 are credited with the first application of the method to biological research. In the course of their investigations in photosynthesis, the respiration of whole plants and

later, of excised leaves was measured by the conductivity method. The carbon dioxide was absorbed in barium hydroxide, originally in the concentration .12N and later reduced to .05N, which was deemed the weakest solution capable of complete absorption. As the formation of barium carbonate precipitate on the electrodes introduced changes in the cell constant, the method was not developed to be one of continuous reading. After the CO_2 had been absorbed in the barium hydroxide, the solution was placed in a tightly stoppered bottle and the resistance determined by a type of dipping electrode, the constant of which was varied with the sensitivity required. With their most sensitive apparatus, one milligram of carbon dioxide produced an observed change in resistance of about 22 ohms. The calibration was carried out by using atmospheric air as a dilute constant mixture of CO_2 and non-reactive gases. The results were plotted graphically and CO_2 equivalents were taken directly from the graph. To increase the sensitivity of the cell, in their latest communication (3), the amount of barium hydroxide solution was decreased from 125 c.c. to 75 c.c.

An extremely sensitive application of the method was described in 1926 by Fenn.(9) To determine the CO_2 output of stimulated nerves, the gas was absorbed in 7 c.c. of a 0.00475M solution of barium hydroxide.

Complete absorption was assured by means of a continuously moving air stream in a closed system. Fenn does not mention any difficulty with changing cell constant due to deposition of barium carbonate on the electrodes, although he does mention that the conductivity change is never quite zero even in the absence of tissue and a small correction must be applied. He suggests this small change may be due to a slow reaction of the alkali with the glass.

Bayliss (10) in 1927 has applied the conductivity method to the absorption of carbon dioxide in sodium bydroxide solution. In this case the increase in resistance is due to the replacement of hydroxyl ion by carbonate ion, which is less motile than the former, so that the conductivity falls as CO_2 is absorbed. Bayliss was concerned with the rapid absorption of relatively large quantities of carbon dioxide (100 c.c. in 10 minutes). The gas was absorbed in .9970 N NaOH and measurements were made with the standard conductivity apparatus. The advantages of absorption in sodium hydroxide solution are discussed in a later section.

In 1927 Raymond and Winegarden (11) applied the method to the estimation of carbon dioxide from fermenting mixtures. Barium hydroxide was used as the absorbing solution and they claim that no difficulty arising from deposition of barium carbonate

in the electrodes was experienced. The authors mention a slight change in the resistance of the solution even in the absence of carbonate, but no attempt is made to explain the phenomenon. A pipette conductivity cell was used to determine the conductivity of the solution after absorption. Using an air flow of about 200 c.c. per minute, Resistance determinations were made 1/2, 1, and 5 minutes after introducing CO_2, and again after 10 to 24 hours. Changes after 1 minute were found to be negligible as long as the solution always contained a relatively large excess of barium hydroxide. The cell was calibrated by producing known amounts of carbon dioxide from sodium carbonate and sulphuric acid solutions.

In a communication of 1935, Newton (12) described the most complete study of the conductivity method to date. The paper included a summary of previous application of the method, as well as a discussion of the theory involved. Newton employed an alternating current Wheatstone's bridge, the alternating current being derived from an oscillating thermionic valve. The sensitivity was increased by use of valve amplification. To preclude the possibility of changing cell constant due to precipitation of barium carbonate on the electrodes, a filter was included in the absorption tube to remove the precipitate from the solution before

it circulated over the electrodes. Estimations may be made in a moving stream of air, so that no accumulation of carbon dioxide occurs. Readings may be taken at short intervals of time, so that changes in the rate of gas production may be detected with a minimum time lag. Calibration was carried out by passing moist atmospheric air through the absorption tube, as in the method of Spoehr and McGee (2).

Steward and Preston,(13), in 1940, have applied the method to the measurement of the respiration of potato discs in their studies on salt accumulation. Platinum electrodes were fitted into modified Reiset absorption towers. Sodium hydroxide solution was used for absorption of the gas and calibration was carried out by the production of carbon dioxide from bicarbonate solution and acid.

The most recent publication on the conductivity method by Clark, Shafer, and Curtis (14), describes a method for obtaining automatic readings. Although the method is of interest, a disadvantage lies in the fact that a very low frequency current is used in the Wheatstone's bridge. It would appear from the publication that a 60 cycle alternating current is employed and such a low frequency introduces errors due to polarization. A frequency of 1000 cycles is generally accepted to be the most satis-

factory for conductivity measurements, as lower frequencies introduce polarisation, while those of too high a frequency are not readily audible by the telephone null-point method.

The preceding account gives a summary of the most important papers dealing with the conductivity method. A brief discussion of the theory of the method is contained in the following section.

Theoretical Discussion.

The most comprehensive discussion of the conductivity method of CO_2 estimation as applied to biological investigation is contained in the previously mentioned paper by Newton. The author is indebted to that source for the following theoretical considerations.

In Newton's communication the following simplifying assumptions are made:

(a) that all salts present are completely ionized in the sense of the term as used by Arrhenius,

(b) that ionic mobilities are unaffected by changes in concentration,

(c) that the law of independent migration of ions holds strictly true, and that no solute is adsorbed on the precipitate.

Let $x_1 \ldots x_2 \ldots x_3$ be the number of ions of different species per c.c.

Let $r_1 \ldots r_2 \ldots r_3$ be the number of ions of different species produced by the addition of one gram of the reacting gas. Note that when ions are removed by the gas, the sign of the corresponding 'r' will be negative. Let 'q' be the weight of the reacting gas introduced into the system of volume 'v'. Then the change in number of ions present (dx) is equal to the change in the amount of gas added (dq) multiplied by the number of ions produced per c.c., per gram of gas, i.e.:

$$dx = \frac{dq.r}{v} \quad \ldots(1).$$

Now if $k_1 \ldots\ldots k_2 \ldots\ldots k_3$ by the specific conductivities of the various ions, the total conductivity will be:

$$c = \sum kx \ldots(2).$$

and $$dc = \sum kdx \ldots(3).$$

therefore $$dc = \frac{dq}{v} \sum kr \ldots(4).$$

so that $\frac{dc}{dq}$ is a constant and equal to $\frac{kr}{v}$. From the above relation:

$$dq = \frac{v.dc}{\sum kr} \quad \ldots(5).$$

If changes in conductivity are measured by means of a Wheatstone's bridge, the sensitivity of the bridge (s) is determined by the relation $s = \frac{dl}{l}$, where dl is the smallest movement of the contact

on the bridge were which can be detected, and l is the length of the wire. It can be shown that $s = \frac{dc}{c}$ so that:

$$dq = \frac{v.\ s.\ c.}{\sum kr} \quad \ldots(6).$$

$$dq = \frac{v.\ s.\ \sum kx}{\sum kr} \quad \ldots(7).$$

From the above relation it is clear that, theoretically, there is no limit in smallness of the amount of gas measureable: dq may be decreased either by decreasing the volume of the absorbant (v) or decreasing the concentration of the absorbant ($\sum kx$). The smallest measureable quantity may be decreased also by increasing the sensitivity of the bridge i.e., decreasing the magnitude of 's' by using a longer wire or extension coils. In practice, however, there is a limit beyond which the volume cannot be reduced or the concentration decreased, so that the sensitivity of this method is largely determined by the efficiency of the absorbing cell.

The theoretical discussion of the method includes cases in which no precipitate is formed, such as absorption of carbon dioxide in soda (Bayliss (10) or cases in which a precipitate removes the gas, e.g., in absorption of carbon dioxide in baryta. Consider the reaction:

$$AB + CD = AD + CB$$

where AB is the substance in solution in the cell,

CD the reacting gas, or its compound with water, and AD is removed by precipitation. Suppose the values of the specific conductivities of the A, B, C, D, ions are k_a, k_b, k_c, k_d respectively. As the B ions remain in solution they do not affect the conductivity, and the ions of AD are removed by precipitation. Hence the number of ions produced by the gas (in the nomenclature used above) is given by $r_c - r_a$.

Hence:

$$\frac{dc}{dq} = \frac{1}{v} \left(k_c r_c - k_a r_a\right).$$

The conductivity will therefore either increase or decrease as $k_c r_c$ is greater or less than $k_a r_a$. A case in which the conductivity increases is exemplified by the estimation of hydrogen sulphide by absorption in silver nitrate:

$$H_2S + 2AgNO_3 = Ag_2S + 2HNO_3$$

As both silver and hydrogen are monovalent $r_{Ag} = r_H$ and the values of k are proportional to the ionic mobilities (u), hence:

$$\frac{k_H}{k_{Ag}} = \frac{u_H}{u_{Ag}} = \frac{330}{56}$$

therefore

$$\frac{dc}{dq} = + \frac{1}{v} \left(\frac{274}{330} \; k_H r_H \right)$$

The positive sign shows the increase in conductivity and the value $\frac{274}{330}$ is proportional to the magnitude of this increase.

A decrease in conductivity is obtained in the absorption of carbon dioxide in soda as used by Bayliss (10):

$$2NaOH + CO_2 = Na_2CO_3 + H_2O.$$

In this case no precipitate is formed, but the water produced is practically un-ionized. In this case

$$r_{OH} = 2r_{CO_3} \text{ and } \frac{u_{OH}}{u_{CO_3}} = \frac{180}{40} \text{ so that:}$$

$$\frac{dc}{dq} = -\frac{1}{v}\left(\frac{8}{9} k_{OH} r_{OH}\right)$$

Absorption in baryta presents an interesting case as neither of the products are ionized:

$$Ba(OH)_2 + CO_2 = BaCO_3 + H_2O$$

in this case $\frac{dc}{dq} = -\frac{1}{v}\frac{415}{360} k_{OH} r_{OH}$

This result can be compared with that obtained with soda, in which:

$$\frac{dc}{dq} = -\frac{1}{v}\left(\frac{320}{360} k_{OH} k_{OH}\right).$$

The baryta method, therefore, is more sensitive than the soda method in the proportion of $\frac{415}{320} = 1.3$. Arising from the discussion it is clear that theoretically, with complete ionization, the relation between change in conductivity and mass of gas added should be linear.

Although the preceding theoretical discussion

by Newton definitely shows the greater sensitivity of the baryta method, there are certain practical considerations which throw a very favorable light on the sodium hydroxide method. Sodium hydroxide has the advantage that no precipitate is formed by the reaction and this does away with the possibility of a changing cell constant from that source, as indicated by some authors. Another advantage of the soda method is that the initial concentration of sodium hydroxide can be made considerably greater than that of baryta, so that for a given cell volume larger quantities of carbon dioxide can be absorbed. In the opinion of the author these two advantages throw the balance of favor toward the sodium hydroxide absorbent, in spite of the decreased sensitivity.

Description of Apparatus

Electrical equipment:

An alternating current Wheatstone's bridge was used to measure the resistance of the cell, the A.C. supply being taken from a General Radio Audio-Oscillator, driven by two 2-volt storage cells. The complete circuit is shown in the accompanying diagram (Fig. 3). The resistance used was a No. 4775 Leeds and Northrup inclosed switch resistance box, capacity 9999 ohms. The metre wire and scale used in preliminary work was

later replaced by a Leeds and Northrup circular slide-wire, with end coils 4.5 times that of the slide wire. The null point was determined with radio headphones of the usual type. Because its construction is similar to that of a condenser, the cell has a certain low capacity and to obtain a sharp null-point, this capacitance of the cell must be balanced out with a variable-capacity air condenser in parallel with the resistance box. Under these conditions a very sharp null-point could be determined. To obtain the clearest note, the oscillator output impedance most closely matched to the initial resistance of the cell was used.

In actual practice, the battery circuit is closed for 2-3 minutes before balancing the bridge to allow the oscillator to warm up. Switch (2) is then closed and an approximate balance is obtained. To obtain a fine balance, switch (3) is closed and the variable resistance is adjusted till the balance is close to 500 on the dial of the slide wire and both resistance and slide wire readings are recorded. The exact resistance is determined by use of the formula:-

$$C = R \frac{4500 \pm A}{5500 \mp A} \qquad \text{(for use with end coils)}$$

where C = unknown

R = resistance

A = dial reading.

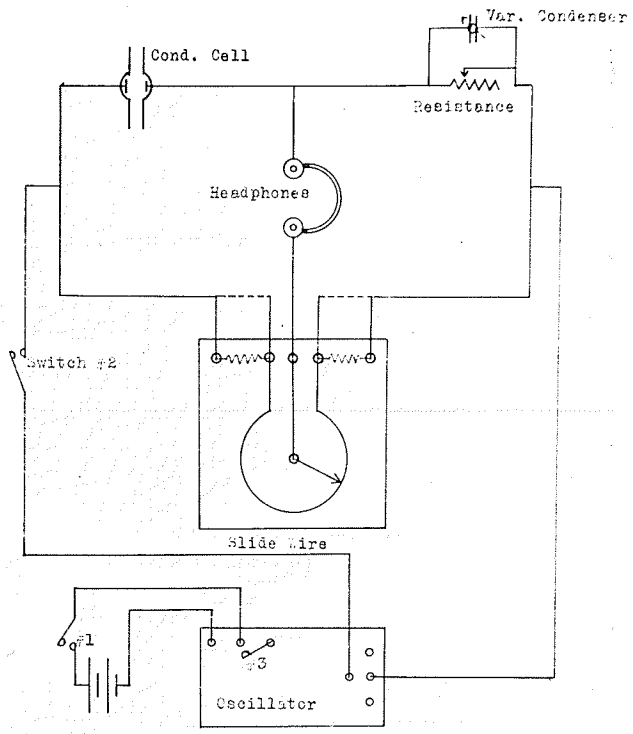


Diagram of Conductivity Circuit.

Fig. 3

The value for $\frac{4500 \pm A}{5500 \mp A}$ is obtained from previously prepared tables supplied with the slide wire and the ratio factor for the dial reading is simply multiplied by the resistance to give the exact value. An example will serve to make this clearer:

In practice, one of the absorption tubes used had an initial resistance of about 600 ohms when filled with 50 c.c. of $\frac{N}{10}$ NaOH solution. Consequently the oscillator output impedance marked 500 ohms was used. The bridge was roughly balanced at 630 ohms and the fine balance was found at 505 on the slide wire dial. From the table the factor for 505 was found to be 1.0020; consequently the correct resistance was 630 x 1.0020 = 631.26 ohms. During the course of the reading the variable condenser was adjusted to give the best null-point. The conductivity cell was immersed in a thermostatically controlled and constantly stirred water bath, maintained at a temperature of 25°C with a temperature variation of about 0.01°C. All experimental work was carried out at this temperature. The regulation of the bath temperature has been shown to be a very important factor in conductivity measurements. The constant temperature bath was electrically grounded to prevent external interference.

The Absorption Tubes:

In the course of the present work two general

types of absorption tube were used, both types giving highly satisfactory results. The tubes are constructed of Pyrex glass tubing to minimize the reaction between the alkali and glass. The first type is illustrated in Fig. 3$_1$. It consists of a glass cylinder about 2cms. in diameter and 40 cms. in length with a drawn-out lead-in tube sealed into one end. In the centre of the tube and with the enlarged end fitting over the extension of the lead-in tube is a tightly wound glass spiral, extending about three-quarters of the length of the tube. The collar of the enlarged end is molded up in three places to allow free passage of the solution into the spiral. Two platinum electrodes are sealed into the opposite sides of the absorption tube, their distance apart being determined by the required resistance of the conductivity cell. Care must be taken to insert the upper electrode well below the surface of the absorbent, to prevent skin effects at the surface. The two side arms inserted at the point of the electrode seals are extended up to the top of the tube and secured there to prevent breakage. Contact is made with the electrodes through a mercury column in the side arms, into which are dipped leads surfaced with mercury amalgam to ensure good contact. In operation the air stream enters at the draw-out inlet tube and is broken up into

small bubbles. As these pass up the spiral, the absorbent is carried up between the bubbles. Thus in passing up the spiral the air bubbles with the carbon dioxide are in contact at all times with a very large, continually changing absorbent surface. This system offers ideal conditions for complete absorption. The air stream also has a very efficient stirring function and the absorbent is kept continually in circulation.

The recurved top of the spiral returns the solution to the body of the tube and the air stream passes out through the top. This tube has a capacity of 50 c.c. of absorbing solution and carbon dioxide from the plant chamber is completely absorbed in this system with an air-flow of 200 c.c. per minute.

The second type of absorption tube is illustrated in Fig. 3II, and by photograph in Fig. 5. In this case the spiral surrounds the central column in which the electrodes are sealed. The tube is filled with a stopcock at the bottom to facilitate emptying and rinsing. The air stream enters through a constricted lead-in at the base of the coil, but the same "broken-column" principle holds. The capacity of this tube is 25 c.c. of absorbent and the rate of flow is the same. As in the first case the following features of design are stressed:

Figs. 3_I & 3_{II}

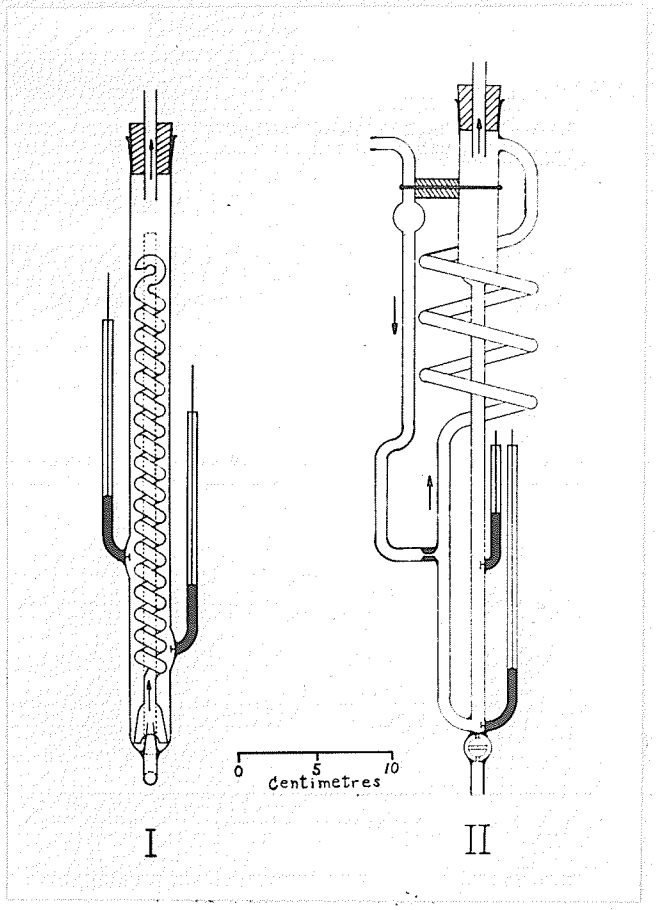
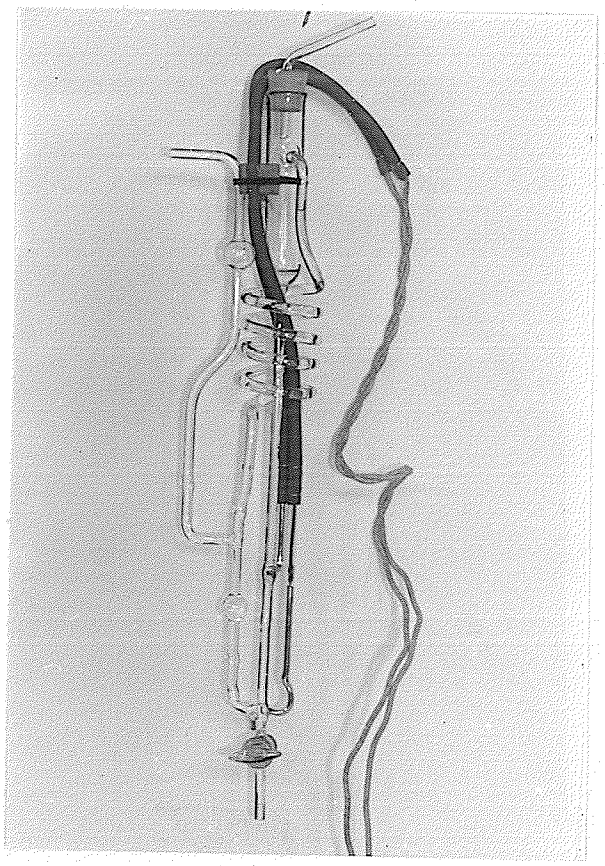


Fig. 4



(1) Efficient absorption

(2) Thorough mixing of solution

(3) Minimum of "dead" solution

(4) Avoidance of skin effects

(5) Compactness

(6) Mechanical strength

The second type of tube is much more sensitive than the first, due to reduction in the volume of absorbent, increase in resistance obtained by spacing the electrodes well apart and decrease in their total surface. Both tubes were designed and constructed by Dr. W. Leach. Preliminary work was done with an absorption tube of the first type, but the second type was used for later investigations owing to its greater sensitivity. The resistance of the absorbent could be determined in the case of the first tube without stopping the air flow, but this was not true in the second case. Because of the small diameter of the tube into which the electrodes are inserted and the rapid stirring action of the air stream, the resistance varied greatly in the second tube while the air stream was passing through it. Consequently the flow was stopped and the solution was allowed three minutes to settle before the resistance was determined.

<u>The Absorbing Solution</u>:

As mentioned previously, sodium hydroxide solution

was deemed to be the most satisfactory absorbent for this work. Preliminary investigations showed that a strength of about N/10 would serve very well. This concentration gives very efficient absorption, yet is dilute enough to give a sufficiently high resistance in the absorption tubes used.

From the equation:

$$2NaOH + CO_2 \quad Na_2CO_3 + H_2O$$

it can be shown by simple calculation that 50 c.c. of N/10 NaOH solution is capable of absorbing 110 milligrams of carbon dioxide. To ensure a surplus of NaOH in the absorbent at all times, calibrations were extended over a range of less than half the possible absorptive power of the solution. The stock solution was prepared from Baker's Analysed Sodium Hydroxide and kept in a tightly sealed container fitted with an automatic burette to deliver the desired amount of solution. The container was thoroughly shaken before the removal of each quantity.

The Calibration of the Cell.

In previous investigations of this nature, two methods have been commonly used in the calibration of the conductivity cell. The first method as applied by Spoehr and McGee (2), is the use of atmospheric air as a dilute constant mixture of carbon dioxide and non-reactive gases. The constancy of its composition is justified by reference to the work of Benedict (15).

Dry air contains about .6 milligrams of CO_2 per litre. To use this method to calibrate conductivity cell over a range of 50 milligrams of carbon dioxide would mean aspirating approximately 83 litres of air through the absorbing solution. Apart from the very tedious procedure, a further disadvantage is the effect of passing such a large volume of "unconditioned" gas through the solution. The effect of this is discussed in detail in a later section but it is obvious that a calibration requiring such a long period allows the possible introduction of a very considerable error due to changes in the solution produced by other than the formation of carbonate.

A second method of calibration which has been used by other authors has been attempted in this investigation with very unsatisfactory results. The method referred to is that of producing carbon dioxide from known amounts of carbonate solution by the action of sulphuric acid. This method is claimed to have been used successfully by Steward and Preston (13) in their calibration covering a range of close to 600 milligrams of carbon dioxide. In the present work the greatest range of calibration is 50 milligrams, so the problem was one of producing quantities of carbon dioxide of less than 10 milligrams. Although several attempts were made, no close agreement of results could be obtained. The difficulty arises from

the fact that, for accuracy, sodium carbonate solutions must be made fairly dilute to contain the required amount of salt. As the carbon dioxide produced is appreciably soluble in the liquid from which it is released, the solution must be heated to drive over all the gas. Complications arose from the application of the heat. Apparently either heat, or some product of the reaction other than carbon dioxide was being carried over and caused inconsistent changes in the conductivity of the absorbent. After several trials this method was abandoned in favor of the following one which gave highly satisfactory results.

A mixture of carbon dioxide and air was prepared by introducing about 40 c.c. of carbon dioxide into 8 litres of air contained in a 9 litre aspirator jar, over 5% sulphuric acid. Carbon dioxide is not soluble in acid solution of this concentration and consequently this solution was used to displace the gas throughout the whole procedure. Samples were taken from the mixture and analysed for CO_2 content by means of a Fisher Precision Gas Analysis Apparatus. All analyses were performed in duplicate and results checked very closely. The gas mixture was then passed through the absorption tube immersed in the bath. The gas was introduced in half litre quantities and the corresponding changes in the resistance of the solution were recorded. A calibration of this type can be completed

in slightly more than one hour and does away with the possibility of errors introduced by methods extending over a long period of time. The resistance-carbon dioxide curve was plotted and was found to be a regular curve, slightly concave to the abscissa. The data and curve for a typical calibration follow.

Calibration of Absorption Tube, Type I.

Capacity of tube = 50 c.c. of approx. N/10 NaOH solution.

Percentage composition of gas mixture.
(average of two determinations) .426%.

Correction of Volume to S.T.P. for temperature 23°C, and Barometric Press. 732 mm.

$$1000 \times \frac{732}{760} \times \frac{273}{296} = 890 \text{ c.c.}$$

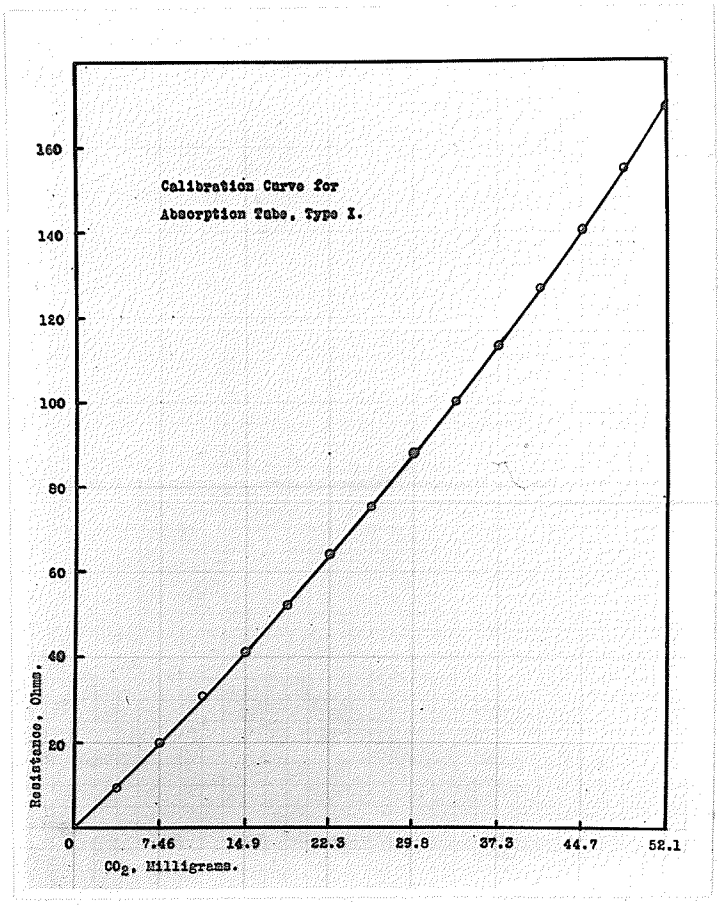
Mixture contains .426% or 3.8 c.c. per litre.

i.e. $\frac{3.8}{22400} \times 44 = 7.46$ Milligrams CO_2 per litre.

Table III. Data for Absorption of Gas Mixture.

Litres of gas	Resistance Box	Dial Setting	Resistance	Change from Zero	Mg. CO_2
0	600	530	600.72	0	0
.5	610	501	610.24	9.52	3.73
1	620	502	620.74	20.02	7.46
1.5	630	505	631.26	30.54	11.20
2	640	508	642.05	41.33	14.95
2.5	650	513	653.38	52.66	18.84
3	660	518	664.82	64.10	22.4
3.5	680	497	676.60	75.88	26.1
4	690	495	688.62	87.90	29.8
4.5	700	504	701.12	100.40	33.6
5	710	514	714.98	113.26	37.3
5.5	730	492	727.66	126.94	41.0
6	740	503	740.89	140.17	44.7
6.5	760	485	755.44	154.72	48.5
7	770	500	770.00	169.28	52.1

The curve for the foregoing data is shown in the accompanying diagram , (Fig. 5). As pointed out previously the volume of solution used is theoretically capable of absorbing 110 mg. of CO_2, but the range of calibration covers only 52.1 mg., allowing a large excess of sodium hydroxide to ensure complete absorption. To obtain data


Calibration Curve for
Absorption Tube, Type X.
160
140
120
100
80
60
40
20
Resistance, Ohms.
0
7.46
14.9
22.3
29.8
37.3
44.7
52.1
CO_2, Milligrams.

from the curve, the latter was drawn on a scale such that readings directly from the chart would equal in accuracy the resistance measurements. In this manner the change in resistance were readily transformed into carbon dioxide equivalents. The calibration of the absorption tube of the second type was carried out in the same manner. Because the curve is not a straight line, an ohm will have a slightly different equivalent value in milligrams of carbon dioxide on different portions of the curve. For examplein the calibration of the absorption tube of the second type it was found that one milligram of carbon dioxide caused a change of 26 ohms for the first quantity of the mixture introduced, while the last milligram of carbon dioxide produced a change of 55 ohms in the absorbing solution. In the most sensitive arrangement developed by Spoehr and McGee (3), one milligram of carbon dioxide produced a change of 21.7 ohms. The greatly increased sensitivity of the second type of absorption tube is due to the reduction of the volume of absorbent to 25 c.c. and the increase of the resistance to an initial value of 3600 ohms. From the calibration data included in Table III, it can be shown that one milligram of carbon dioxide produces a change of approximately 3 ohms. Theoretically, for the same absorbent, decreasing the volume by half should double the sensitivity and increasing the resistance six times should increase the sensitivity by the same amount i.e. the initial

sensitivity of the second absorption tube should be twelve times that of the first. Actually one milligram of carbon dioxide in the second tube produces $\frac{36 \text{ ohms}}{3 \text{ ohms}}$, or 12 times the change produced in the first tube. Although the figures stated are approximations, they are sufficiently accurate to prove the theoretical expectation.

The Respiration System.

The respiration chamber consisted of a small wide-mouthed jar, fitted with two glass tubes, one of which extended well down into the jar. These were inserted through a tight-fitting cork. Preceding the respiration chamber was a water bubbler with an internal spiral similar to that of the first type of absorption tube. It is necessary to moisten the air passing through the respiration chamber to prevent drying of the plant material. The outlet tube was connected directly to the absorption tube. Air was drawn through the system by means of suction from a filter pump. The air was brought in from outside to eliminate the presence of sulphur dioxide, or other laboratory gases which might pass through the system with unpredictable consequences to the respiring material and the absorbent. It was carried by means of large bore glass tubing to a bank of U-tubes filled with fairly course soda-lime. Before passing into the

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water bubbler, the air was bubbled through barium hydroxide solution to prove the absence of traces of carbon dioxide. A bulb was installed before the absorption tube to prevent any liquid from being carried over with the air stream. A variable flow-meter using a glass resistance of the type described by Gregory (16) was installed after the absorption tube and the air flow was adjusted and maintained at 175 c.c. per minute. Thus the air in the respiration chamber is completely changed several times a minute, preventing any accumulation of carbon dioxide. All glass tubing connections were fitted closely together and sealed with heavy pressure tubing wired to the glass, to prevent leakage of carbon dioxide through the rubber. This precaution was taken due to the fact that early in the experimental work trouble of this nature was experienced. In the original system the carbon dioxide was removed from the air stream at the point where the tubing came from outside the laboratory. After passing through the soda-lime it was carried several feet through glass tubing which was joined with the common gas-line rubber tubing. It was soon discovered that quantities of carbon dioxide sufficient to cause very significant errors were diffusing through the rubber tubing into the air line. To eliminate this difficulty the soda-lime tubes were installed in the system at a point just before the air stream entered the first bubbler in the water bath.

At the onset of respiration experiments, several blank runs were made in the absence of carbon dioxide to determine the constancy of the apparatus. Runs were extended over a period of at least 36 hours and readings were taken hourly as far as possible. Under these conditions a gradual decrease in the resistance was observed, indicating an increase in the concentration of the absorbent, possibly due to the removal of water by the air stream. The changes were plotted and were not found to be sufficiently regular to allow a correction to be applied. The system was then changed to include another bubbler of the same type as that containing the water. This bubbler was placed between the water bubbler and the respiration chamber. About 50 c.c. of sodium hydroxide solution of the same stock as that in the absorption tube were placed in the bubbler. The reason for this procedure was to "condition" the air stream by bubbling it through a blank absorption tube, so that changes produced would not be recorded. Thus the vapor pressure of the air stream is in equilibrium with the solution in the second absorption tube before passing through it and consequently produces no further changes in the absorbing solution. The proof of this theory was shown experimentally by further runs. It was found that the resistance no longer decreased as before, but remained fairly constant. The term "fairly" constant is used because a slight creep in the opposite direction was detected. Readings over

a long period of time showed that the resistance was increasing very slightly, even in the absence of carbonate. The changes in resistance were plotted and found to be quite regular. Consequently a correction could be readily applied and no further attempt was made in this investigation to determine the cause of the creep. This slight change in the resistance of the solution is mentioned by both Raymond and Winegarden (11) and Fenn (9); the latter suggests that it may be due to a slow reaction between the alkali and the glass. The solution in the extra sodium hydroxide bubbler was changed frequently to keep the normality close to that of the solution in the conductivity cell. The magnitude of the error was redetermined by a blank run following any change in the system to check its constancy.

As the apparatus is not automatically recording, and the runs were to be carried over 24 hour periods, it was necessary to use two samples of material, started 12 hours apart to get a complete record. In practice one sample of barley was placed in the respiration chamber at 8.30 A.M. and readings were taken at hourly intervals for 12 hours. At that time a fresh sample was started and readings were not taken till 12 hours later, i.e. 8.30 A.M. the following morning and were continued for 12 hours to complete the 24 hour run. The two curves were plotted together.

The transition in practically all cases was quite smooth and the method was very satisfactory. In the final experimental arrangement, by the use of two-way stop-cocks, duplicate respiration chambers and by-passes, the air stream could be diverted in a number of different paths. A diagram of the system and its possibilities is shown in the accompanying illustration (Fig. 6). In this way the grains may be brought to any desired period of growth under constant experimental conditions before starting a record of the respiration. This effected a considerable saving in absorbent, as carbon dioxide was not being absorbed during periods when no readings were being taken. It also obviated the necessity of disturbing the apparatus during determinations.

For each experiment two samples of barley of 30 grains each were selected from the storage container. Only plump grains in good condition were used, each sample weighing about 1.15 grams. The grains were placed in the respiration chamber on three thicknesses of filter paper and thoroughly wetted with a surplus of distilled water. The excess water was then pipetted off till the grains were left half submerged. When the complete apparatus had been placed in the bath, CO_2 free air was passed through the system for 15-20 minutes to remove all traces of carbon dioxide. The first resistance determination was made after the

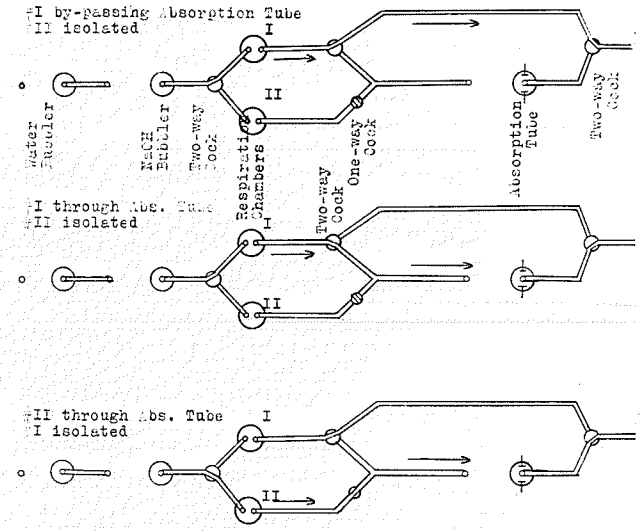

Diagram of Duplicate Chamber System,
Showing Possible Arrangements.

Fig. 6

apparatus had been in the bath for half an hour to allow the absorbing solution to reach the bath temperature. At the end of each 24 hour run the percentage germination was determined. Growth of the individual grains of the sample proceeded at a fairly uniform rate. In 24 hours the rootlets which were generally three in number were about 4-5 mm. in length. In the normal grain the coleoptile had not yet emerged at this stage.

Experimental Results.

The respiration rates of two varieties of barley during water absorption and germination have been investigated by the described method. The rates of respiration for three samples of the normal grain of the variety O.A.C.21 are shown graphically in Fig. 7, and numerical data relating to these experiments and three others are given in Tables IV and V. Examination of the tables and graphs reveals that the respiratory activity is characterized by three more or less well marked stages in each case. The first stage is marked by a fairly rapid rise in the rate of respiration, starting close to the second hour after contact with water and continuing at a uniform rate until the 12th to 14th hour, varying with the experiment. The second stage is introduced by a decrease in the rate of respiration indicated by a general flattening of the curve. The degree of clarity of this decrease varies considerably from experiment to experiment, but some trace of it is

TABLE IV Data for the Respiration of Normal Grain of O.A.C.21.

Expt. No. 12		Expt. No. 13		Expt. No. 14	
Germ'n Time, Hr.	Resp'n Rate	Germ'n Time, hr.	Resp'n Rate	Germ'n Time, hr	Resp'n Rate
1	.08	2.5	.11	2.5	.09
2	.12	5.5	.24	4	.09
4	.22	7	.26	5.5	.18
6	.26	8	.36	7	.32
7	.31	9	.37	8.5	.35
8	.36	10	.40	9.5	.52
10	.45	11	.53	11.5	.58
11	.58	13.5	.62	13.	.59
12	.66	14.5	.66	14.	.62
13.5	.78	15.5	.64	15.	.66
15	.88	16.5	.66	16	.73
16	.93	18.5	.74	17	.81
17	.97	19.5	.72	19	.91
18	1.03	21.5	.91	21	.99
21	1.22	22.5	.96	22	1.03
22	1.32	23.5	1.03	23	1.06
23	1.36	25.	1.07	24	1.16
24	1.40	26.	1.09		
96% Germination		73% Germination		78% Germination.	

TABLE V Respiration Data for the Normal Grain of O.A.C.21

Expt. No. 15		Expt. No. 16		Expt. No. 17	
Germ'n Time, hr.	Resp'n Rate	Germ'n Time. hr.	Resp'n Rate	Germ'n Time. hr	Resp'n Rate
3	.13	2	.05	4	.65
4	.18	3	.14	5	.13
6	.22	4	.21	6	.17
8	.30	6	.22	8	.38
9	.42	7	.33	9	.49
10	.45	9	.43	10.5	.62
11	.53	10	.49	12	.75
13.5	.65	12	.68	15	.80
14.5	.69	13	.85	17	.88
15.5	.68	14	.97	18	.93
16.5	.69	15	1.02	19	1.09
17.5	.67	18	1.06	21	1.21
18.5	.77	19	1.11	23	1.38
19.5	.76	20	1.24		
21.5	.94	21	1.30		
22.5	.98	22	1.42		
25	1.10	23	1.46		
26	1.12				
75% Germination		97% Germination		94% Germination	

generally present. The onset of the third stage is marked by a maintained increase in the rate of respiration, and from this point on to the end of the experiment no further significant change occurs. The higher rates of respiration in the second and third stages of Experiments No. 16 and 21 may be correlated with the fact that their germination percentage as shown in the tables approaches 100%, while that of Experiment No. 15 is only 75%.

With a view to exhibiting varietal differences, the rate of respiration of the normal grain of the variety Wisc. 38 was investigated in the same manner. The variety O.A.C.21 is a more active malting barley, having greater diastatic and proteolytic properties than the variety Wisc. 38, and on these grounds it was expected that varietal differences might be detected in their rates of respiration. The graphs showing the rates of respiration of three samples and the numerical data relating to these and two other experiments are given in Tables VI and VII. This variety exhibits the same sequence of respiratory stages which were demonstrated by the variety O.A.C.21. There were no obvious varietal differences distinguished from the respiratory data, although it was observed that the total root growth of the Wisc. 38 at 24 hours was less than that of the other variety.

To determine the effect of the hull on respiration

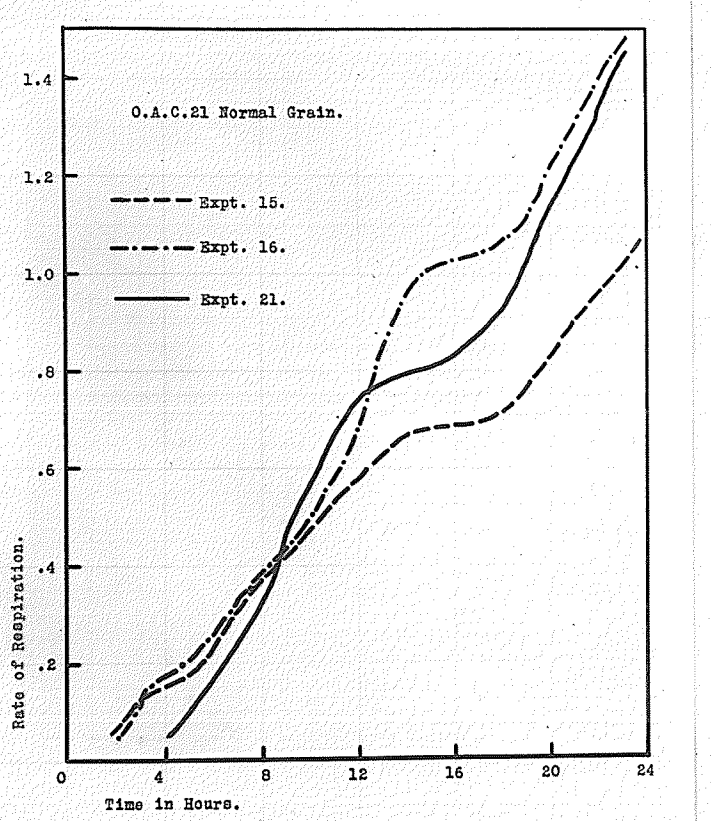
O.A.C.21 Normal Grain.
Expt. 15.
Expt. 16.
Expt. 21.
Rate of Respiration.
Time in Hours.
0
4
8
12
16
20
24
.2
.4
.6
.8
1.0
1.2
1.4

Fig. 7

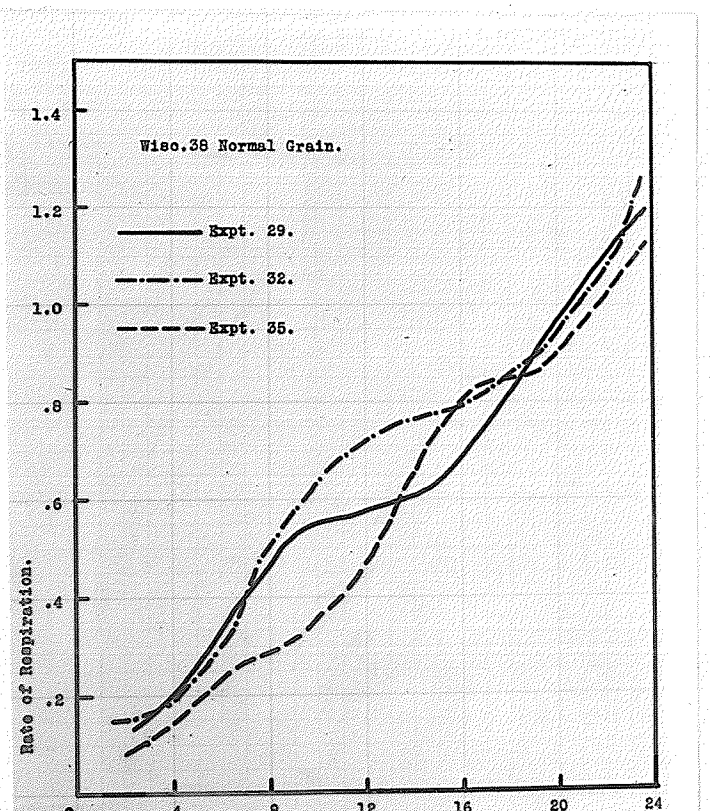
Wisc.38 Normal Grain.
Expt. 29.
Expt. 32.
Expt. 35.
Rate of Respiration.
Time in Hours.
0
4
8
12
16
20
24
.2
.4
.6
.8
1.0
1.2
1.4

Fig. 8

TABLE VI Respiration Data for Normal Grain of Wisc.38

Expt. No 27		Expt. No. 29		Expt. No. 32	
Germ'n Time, hr.	Resp'n Rate	Germ'n Time. hr.	Resp'n Rate	Germ'n Time, hr	Resp'n Rate
3	.13	2.5	.18	1.5	.15
5	.19	3.5	.13	2.5	.16
7	.28	4.5	.26	4.5	.22
8	.30	5.5	.26	5.5	.26
10	.32	7	.40	6.5	.33
11	.34	8.5	.48	7.5	.48
12	.51	9.5	.56	8.5	.53
13	.54	10.5	.55	9.5	.59
14	.65	11.5	.56	10.5	.67
16	.71	13	.59	12	.72
18	.79	15	.63	13	.75
20	.86	17	.75	14	.77
21	.93	18	.80	15	.75
22	1.00	19	.91	16	.79
23	1.02	20	.94	18	.85
24	1.10	21	1.07	19	.89
		22	1.10	20	.97
		23	1.17	21	1.00
		24	1.19	23	1.21
75% Germination		85% Germination		85% Germination	

TABLE VII Respiration Data for Normal Grain of Wisc. 38

Expt. No. 35		Expt. No. 33	
Germ'n Time, hr.	Resp'n Rate	Germ'n Time, hr.	Resp'n Rate
2	.08	2.5	.09
3	.12	4.5	.26
4	.12	6.5	.48
5	.21	7.5	.51
6	.21	8.5	.55
8	.28	9.5	.61
9	.31	10	.58
10	.35	12	.62
11	.40	14	.66
12	.46	15	.74
13.5	.62	16	.80
14.5	.72	17	.82
15.5	.76	18	.87
16.5	.85	20	.97
17.5	.83	22	1.02
18.5	.84	23	1.09
19.5	.90	24	1.13
21.5	1.02		
24	1.12		
80% Germination		80% Germination	

the lemma and palea were removed from several grams of both varieties and their rates of respiration were investigated as before. In this case a most remarkable increase in the rate of respiration was exhibited by both varieties of barley. The variety O.A.C.21 in this case showed the more intense respiratory activity. The curve for the hulled grain as shown by Expt. No. 43 in Fig. 9, rises steeply and continues at an even rapid rate, until at 24 hours the grain is respiring at more than twice the rate of normal grain. The curve is characterized by an almost complete absence of the sequence of periods noted in the normal grains. Detailed information related to this curve and its duplicate are to found in Table VIII. For purposes of comparison the respiration curves of two normal samples of O.A.C.21 are included in Fig. 9. (Expts. No. 15 & 21). The respiration curve for the hulled grain of Wisc.38 is also included in Fig. 9, (Expt. No. 49). Although the respiratory activity in this case is not as intense as that of the other variety, it is still far above that of the normal grain at 24 hours. Further data for this experiment and its duplicate are found in Table VIII.

The lemma was removed from a sample of the variety of O.A.C.21 and two respiration determinations were made. The data for these is included in Table II and the curve of one of them (Expt. 45) is shown in

TABLE VIII Respiration Data for Hulled Grains of O.A.C.21 and Wisc. 38.

Expt. No. 41 O.A.C.21		Expt. No. 43 O.A.C.21		Expt. No. 51 Wisc. 38		Expt. No. 49 Wisc. 38	
Germ'n Time, hr.	Resp'n Rate	Germ'n Time, hr.	Resp'n Rate	Germ'n Time, hr.	Resp'n Rate	Germ'n Time, hr	Resp'n Rate
2.5	.32	2.5	.13	1.5	.14	3	.14
4.5	.36	4.5	.17	2.5	.20	4	.22
5.5	.41	5.5	.32	4.5	.24	5	.37
6.5	.57	6.5	.52	5.5	.38	6	.52
7.5	.70	7.5	.60	6.5	.43	7	.58
8.5	.90	8.5	.70	7.5	.56	8	.72
9.5	1.07	9.5	.90	8.5	.60	9	.77
10.5	1.34	10.5	1.09	9.5	.76	10	.83
13.5	1.52	11.5	1.22	10.5	.84	11	.86
15	1.60	13.5	1.43	11.5	.88	12	.88
16	1.70	14.0	1.66	12.5	.92	13	.91
17	1.86	16.5	1.86	13.5	.94	14	1.00
18	2.04	17.5	1.98	15.5	.96	15	1.14
19	2.07	18.5	2.17	16.5	1.03	16	1.24
20	2.16	19.5	2.19	17.5	1.22	17	1.30
21	2.23	20.5	2.25	18.5	1.27	18	1.36
22	2.24	21.5	2.43	19.5	1.29	19	1.45
23	2.49	22.5	2.55	20.5	1.53	20.5	1.55
24	2.63	23.5	2.66	21.5	1.72	22.5	1.67
				24.	1.90	24	1.78
97% Germination		100% Germination		97% Germination		97% Germ.	

TABLE IX Respiration Data for the Grain of O.A.C.21 with Lemma Removed

Expt. No. 45		Expt. No. 47	
Germ'n Time, hr.	Resp'n Rate	Germ'n Time, hr.	Resp'n Rate
1.75	.07	1	.16
2.5	.14	2	.15
3.5	.16	3	.18
4.5	.24	4	.23
5.5	.24	5	.45
6.5	.36	6	.51
7.5	.35	7	.52
8.5	.50	8	.64
9.5	.66	9	.82
10.5	.68	10	.87
13.5	.86	12	.93
14.5	1.06	13.5	.98
15.5	1.14	14.5	1.02
16.5	1.25	15.5	1.14
18	1.38	16.5	1.21
19	1.51	17.5	1.21
20	1.60	18.5	1.46
22	1.78	19.5	1.64
23	2.08	20.5	1.62
		22	1.92
		24	2.18
97% Germination		100% Germination	

Fig. 9. In this case the respiration rate increases rapidly but steadily with no distinct periodicity, midway between the rates of the completely hulled grain and that of the normal grain.

To check the accuracy of the conductivity method of measuring respiratory intensity, three experiments were carried out by the katharometer method. The author is indebted to Dr. W. Leach for the data obtained by this method. One grain of hulled O.A.C.21 barley was placed in the katharometer and the respiratory activity is shown in Fig. 9 (Expt. 60). The curve checks very closely with that of Expt. 43, obtained by the conductivity method. The two other experiments, Nos. 65 and 70, were conducted with the normal grain of O.A.C.21. In the case of Expt. No. 65 germination was not normal. After 48 hours the only indication of germination was the appearance of the chit at the end of the grain. The respiration curve showed an initial increase which was lower than, but similar in trend to the normal curves obtained by the conductivity method. However, after 17 hours the rate fell slightly and remained constant for the duration of the experiment. Germination was quite normal in the case of Expt. No. 70. During the first period the curve is similar to that of the normal grain obtained by the other method. At 12 hours the curve flattens off, falls

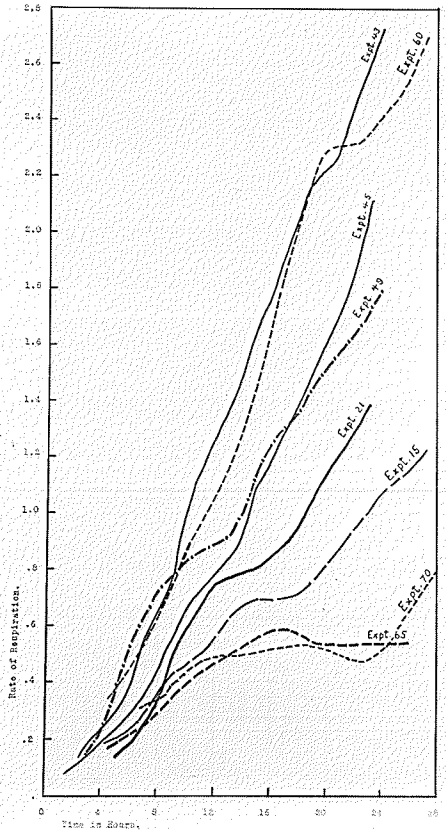
Rate of Respiration.
Time in Hours.
Expt. 40
Expt. 5
Expt. 19
Expt. 21
Expt. 15
Expt. 20
Expt. 65

Fig. 9

TABLE X Respiration Data for the Normal and Hulled Grain of O.A.C.21 Obtained by the Katharometer Method.

Expt. No. 60 Hulled Grain		Expt. No. 65 Normal Grain		Expt. No. 70 Normal Grain	
Germ'n Time, hr	Resp'n Rate	Germ'n Time, hr.	Resp'n Rate	Germ'n Time, hr.	Resp'n Rate
4.5	.34	4.5	.19	7.5	.32
7.5	.58	7.5	.26	10.5	.46
10.5	.92	10.5	.42	13.5	.49
13.5	1.22	13.5	.49	16.5	.52
16.5	1.79	16.5	.59	19.5	.52
19.5	2.29	19.5	.53	22.5	.47
22.5	2.31	22.5	.58	25.5	.63
25.5	2.51	25.5	.53	28.5	.88
28.5	2.85			31.5	1.10
				34.5	1.27
				37.5	1.48

slightly and at 28 hours proceeds to rise quite rapidly. Although it is considerably delayed, the third period proceeds with a trend that is almost parallel to that of Expts. No. 21 and 15, obtained by the conductivity method. The significance of these curves is discussed in a later section. Additional information relating to the katharometer curves is found in Table X.

All respiration data are expressed as milligrams of carbon dioxide per gram fresh weight per hour of grain.

Discussion of Results

I. Water Absorption

The water absorption curves for barley shown in Figs. 2a and 2b lead to some interesting speculations concerning the process of water absorption. While all curves exhibit the same general form, there are some significant differences in the rate at which they approach equilibrium which are worthy of mention. Although different varieties of barley were employed throughout, the data for the normal grain are in reasonable agreement with those obtained by Brown (5) and Wolfe (7). Brown used the variety caerulescens, while Wolfe investigated the variety White Hulless. In the latter case the initial portion of the curve is less steep, but shows complete agreement at 12 hours. The data obtained by Pickler (6) are considerably higher than those obtained for the normal grain of the varieties investigated in this research. This difference is also pointed out by Wolfe, who worked with the same variety of barley as Pickler. It may be pointed out that reasonable allowance must be made for differences in material and methods.

Compared to the normal grain of O.A.C.21, the

water absorption curve for the normal grain of Wisc.38 (Fig. 2b) tends to rise more steeply for the first few hours, after which it falls below the former and parallels it for the remainder of the period. The difference in the absorption rates of the two varieties is not sufficient to be of significance.

The complete removal of the hull does produce a significant difference. In this case a marked rise in the rate of water absorption is observed. The curve, (Fig. 2b) shows a rapid initial increase and a tendency to approach equilibrium earlier in the period. The explanation of this difference probably lies in the damage suffered by the testa and pericarp in the removal of the palea, as pointed out in a previous section. In this case small portions of the semi-permeable membrane lying beneath the testa and pericarp have been removed and the endosperm tissue is in direct contact with the water. Thus the dry storage tissue can readily absorb water without the retarding action of the pericarp, testa and semi-permeable membrane.

Two other cases of increased absorption are shown in Fig. 2a. Although it is purely coincidence, the data for the killed grain and the normal grain in contact with water fall on the same curve. As described previously, the grains were killed by heat before the experiment. After this treatment it was

observed that the grains take up water more rapidly than the normal grain, although their original water content was the same. The cause of this increase in absorption is open to question, but it is quite possible that the heat brought about some change in the permeability of the covering of the grain, or in the absorptive power of the endosperm. These results confirm the findings of Brown (5) who states that the semi-permeable layer is a non-living membrane. If the layer were a living tissue, the death of the cells would be expected to cause a much more marked change in the rate of water absorption, rather than a slight increase with a similar trend.

The case of barley absorbing water under conditions satisfactory for germination is the other interesting example of increased absorption. As pointed out previously all other experiments were carried out with the grain completely immersed in the water and it was suggested that the germination may be retarded due to lack of oxygen. The case in which the water absorption is increased when conditions favorable to germination are provided supports the belief of Steward (18) that absorption is closely connected with respiration. The latter has shown that the salt absorption of storage tissue is increased when the solution containing the tissue is aerated. Another factor which may account for some of the increase in water content is the formation of water

in the process of respiration, in which glucose is broken down to form carbon dioxide and water.

The curve showing the rate of water absorption of barley from a saturated atmosphere (Fig. 2b) is also of interest from a point of view of storage. After 30 hours exposure the water content had risen from the safe storage content of 10.9% to more than 21%. After 10 days the water content was 34%. Under favorable conditions of temperature to initiate an increase in respiration, barley in storage exposed to excessive humidity will readily start to heat.

II Respiration

The marked periodicity of the respiratory activity of the normal grain of barley opens a new field of speculation. Up to the present time respiration data on the early stages of germination are practically non-existent. In most cases workers have dealt with the early stages of the young seedling to obtain data related to malting or plant nutrition. It is interesting to note that a sequence of stages similar to those undergone in barley is discussed in a recent communication by Leach (20) dealing with the respiratory behavior of wheat. Although there is no basis for assuming that the respiratory process is identical in barley and wheat, because of their systematic proximity and morphological similarity, a likeness in their

physiological behavior would not be entirely unexpected. One very obvious difference between the two grains is the absence in barley of the prolonged initial stage described by Leach. In the case of wheat there appears to be a rapid initial rise in the rate of respiration from the resting seed to a low maintained level which continues until the conclusion of the first stage, marking the end of the available substrate. In the case of barley, the respiration rate rises steadily after the second to fourth hour in contact with water. The data for the respiration rate of the dry grain of barley given by Nielsen (19) are .006 - 7 mg.CO_2/gram/hour. Assuming these values to be approximately correct, after contact with water there must be a very rapid initial rise in the rate of respiration which is not recorded due to the limitations of the apparatus. The katharometer data shown by Expts. No. 65 and 70 although insufficient in number to be conclusive, also indicate that the initial period is much shorter in barley. Therefore the first stage described for barley would correspond to the second for wheat. From this point on the agreement is quite close. With the rapidly increasing water content, mobilization of substrate by the hydrolysis of food reserves of the endosperm causes the rapid increase described as the initial stage for barley.

The saturation of the existing oxidizing system as in the case of wheat is suggested as the cause of the dropping of the respiration rate described as the second stage for barley. The end of this stage which is marked by a steady and maintained increase in respiration is explained again as in the case of wheat by the development of new centres of respiratory activity in the form of meristems.

While it is suggested in the preceding discussion that the respiratory processes occuring in wheat and barley may be very similar, it must be made clear that at the present stage of investigation the absence of definite information concerning many related factors allow only tentative theories to be put forward. For example, it is difficult to correlate the effect of the removal of the hull on respiration to the aforementioned theory. Consider for example the respiratory data and curves for Experiments 43 and 60, which show close agreement yet were obtained by entirely different methods. An examination reveals that the initial rate of respiration is maintained through the course of the whole experiment, with the exception of a small variation close to the 20 hour mark, which has not been sufficiently investigated to allow of any theorizing on this point. Considered from this aspect, it would appear that the periodicity observed in the normal grain was caused by either

mechanical resistance to development offered by the hull, or its resistance to the diffusion of carbon dioxide and oxygen to centres of respiratory activity. The effect of the seed covering in retarding the respiration of germinating sweet pea seeds has been demonstrated by Leach (21). The removal of the hull eliminates this resistance and the development as indicated by the respiration proceeded with no variation. A similar explanation applies to the respiration of the grains in Expt. No. 45, from which the lemma was removed with a corresponding loss in the periodicity.

The three experiments performed with the katharometer serve to illustrate the advantage of using a method which reveals the respiratory story of the individual grain. The variation in the rate of respiration close to the 20 hour period of the hulled grains in Expt. 43 and 60 may be taken as examples. The katharometer experiment (No. 60) shows a very distinct decrease in rate for three hours, followed by an increase to the previous rate which is maintained for the duration of the experiment. While the conductivity experiment (No. 43) shows this variation, it is not nearly so distinct as in the record of the individual grain. The respiration rate obtained for a sample of grains as

in the conductivity method can only be the average value as the development of the individual grains cannot be identical. The changes in the respiration rate are masked by those grains whose development is either more or less rapid than the normal. In the case of reduced or abnormal germination in a sample, the calculated rate is proportionately lower than the actual because it must be based on the total fresh weight of the grains, although some of these are not adding to the total carbon dioxide produced. An example of abnormal germination with a reduction in the rate of respiration in the third stage is exhibited by Expt. No. 65, Fig. 9.

In conclusion it may be pointed out that the investigations described in this thesis are only an introduction to the respiratory behavior of germinating barley. Consequently the theories advanced to explain the variation in the rates of respiration of the normal, semi-hulled and hulled grains can only be proved or disproved by further experimental work.

Summary

The water absorption of the varieties of barley O.A.C.21 and Wisc. 38 have been investigated by the centrifuge method at 25°C.

Data and graphs are provided to show the effect of various experimental treatments on the rate of water absorption of barley.

A discussion of the results and their significance is included in the text.

The development of the conductivity method as applied to the estimation of the carbon dioxide output of germinating barley has been described.

By the use of this method the respiratory behavior of the varieties of barley O.A.C.21 and Wisc. 38 has been investigated during the first 24 hours of germination at 25°C.

The respiration curves of the normal grains of both varieties are characterized by three fairly well marked stages. These stages are (1) a fairly rapid increase in the rate of respiration starting after about 2 hours in contact with water, (2) a more or less constant rate of respiration beginning about the twelfth hour, but varying considerably in point of origin and duration with the experiment, (3) a further increase in the rate of respiration which is maintained

for the duration of the experiment.

No marked respiratory differences between the two varieties were observed despite their differences in malting properties.

The respiration rates of the grains with the hulls completely or partially removed have also been determined.

The complete removal of the hull causes a very marked increase in the rate of respiration and the periodicity exhibited by the normal grains is absent. Similar results are obtained by the removal of the lemma, although the increase is not so marked.

The respiratory behavior of barley is discussed and compared with that of wheat.

Tentative theories are suggested to account for the periodicity of the respiration of the normal grain and its absence in the hulled and semi-hulled grain.

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