LVI.—*Experiments on Germinating Barley.*

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THESE experiments were undertaken with the object of throwing some light on the source of the carbonic anhydride produced during the germination of barley, the observations being limited to the earlier stages of germination as carried out in the process of malting. It was proposed, at the same time, to ascertain whether atmospheric nitrogen takes any active part in the germination, and to determine, as accurately as possible, the amount of oxygen absorbed by the grain as compared with the quantity of the same element excreted in the

form of carbonic anhydride. As a result of the inquiry one fact has been elicited, which is of some importance; for as will be seen, it indicates the probable nature of the body, which by its decomposition during germination furnishes the carbonic anhydride.

1. As is well known a considerable quantity of carbonic anhydride is produced during the germination of barley. I made a few preliminary experiments to ascertain the amount of this gas produced, with the main object of finding out the apparatus most suited for the natural germination of the grain during a period of about ten days. It may be as well to notice in passing a few of the results obtained in these preliminary experiments.

The quantity of carbonic anhydride produced varies much under different conditions of temperature and moisture. A moderate elevation of temperature considerably increases the amount of carbonic anhydride formed. The same effect is produced by an increase in the amount of moisture present in the grain. Thus in two experiments on the same barley, germinated at the same temperature, but with different quantities of moisture, the following results were obtained:—In No. 1, 20·17 grams of barley, sprinkled with water during growth, produced 668·8 c.c. of CO_2 ; in No. 2, 20·01 grams of barley (germinated without any sprinkling) produced 596·9 c.c. of CO_2 . Again, two experiments on the same barley with equal quantities of moisture, but grown at different temperatures, furnished the following figures:—In No. 1, 19·88 grams of barley were grown at a temperature of $61\cdot5^\circ \text{F.}$, and produced 775·6 c.c. of CO_2 ; in No. 2, 20·17 grams of barley were grown at a temperature of $57\cdot4^\circ \text{F.}$, and gave 668·8 c.c. of CO_2 .

The total quantity of carbonic anhydride produced, varied in all the experiments (made on about 20 grams of barley in each case) from 1·1679 gram = 592·5 c.c. to 1·7107 gram = 867·8 c.c. From this it can be readily calculated that the weight of carbon oxidised to CO_2 varies from 1·55 per cent. to 2·18 per cent. of the weight of barley employed.

The effect of temperature on the amount of carbonic anhydride produced is not the same in all stages of germination; the point at which an increase of heat fails to produce an increase in the quantity of carbonic anhydride is lower at an early stage of germination than at a later stage. I intend shortly to investigate this point more thoroughly.

2. *Does Atmospheric Nitrogen play any active part in the Germination of Barley?*

Some authors have asserted that the nitrogen of the air plays some part in the chemical changes effected in growing barley during ger-

mination. I quote the following passage from G. J. Mulder's *De la Bière*, p. 107:—"Dans la germination une petite quantité du gaz azote de l'air ambiant est absorbée, et De Saussure n'attribue pas cette absorption à une simple cause physique." But this opinion is not generally admitted, and I therefore thought it well to make several careful experiments to determine the point. According to my experiments there does not appear to be any absorption of nitrogen, but if there is, the amount absorbed is so minute as to lie within the limits of experimental error. The experiments were conducted in the following way:—A weighed amount of steeped barley was placed in the flask C (Fig. 1). This flask was divided into mm. on the neck and carefully calibrated. The flask was inverted, with its mouth below the surface of some mercury (B), at the bottom of a large beaker (A) filled with water. T is a thermometer. A small plug of platinum wire (P) of known weight was placed in the neck of the flask to prevent the corns of barley from falling down to the surface of the mercury. The specific gravity of the barley and of the platinum was determined, so that their volume might be deducted from that of the air in the flask. As soon as the apparatus was arranged, the height of the mercury column was read off on the neck of the flask, the barometer and thermometer being observed at the same time; allowance was, of course, made for the column of water above the surface of the mercury in the beaker.

Fig. 1.

At the end of the experiment the same observations were again made, and a portion of the air in the flask was withdrawn for analysis. The following are the results of three experiments performed with this apparatus:—

No. 1. 4.993 grams of Barley.

Vol. of air at commencement of experiment ...	} 98.72 c.c. containing 79.04 per cent. N = 78.03 c.c. N.		
Vol. of air at end of experiment		97.69	" " 79.55 " = 77.72 "
Decrease....	1.03	Decrease....	0.31
9.128 c.c. of CO ₂ were produced.			

No. 2. 5.352 grams of Barley.

Vol. of air at commencement of experiment ...	{	82.50 c.c. containing 79.04 per cent. N	= 65.21 c.c. N.
Vol. of air at end of experiment	{	81.50 " " 80.33 " "	= 65.47 " "
Decrease....		1.00	Increase.... 0.26
4.018 c.c. of CO ₂ were produced.			

No. 3. 5.345 grams of Barley.

Vol. of air at commencement of experiment ...	{	80.50 c.c. containing 79.04 per cent. N	= 63.69 c.c. N.
Vol. of air at end of experiment	{	79.61 " " 80.09 " "	= 63.76 " "
Decrease....		0.89	Increase.... 0.07
4.118 c.c. of CO ₂ were produced.			

From these results it will be seen that the total quantity of nitrogen in the confined volume of air is sometimes increased, and sometimes diminished to a very small extent. The air used in the experiments was not previously analysed, but was assumed to contain the average amount of nitrogen, *i.e.*, 79.04 per cent. by volume. The result of the experiments certainly seems to prove that nitrogen takes no active part in the germination of barley.

It will be seen that the determination of this point was a necessary prelude to the experiments about to be described.

3. *The Absorption of Oxygen during Germination.*

It has been conjectured, and in certain cases proved, that more oxygen is absorbed by germinating seeds than is required for the production of the carbonic anhydride. In Mulder's *De la Bière* (p. 106) the following passage occurs:—"De Saussure pense que, bien que le fait ne puisse pas toujours être sensible dans les expériences faites dans l'air atmosphérique, la semence s'assimile toujours dans la germination une certaine quantité d'oxygène que l'on ne peut pas retrouver dans l'acide carbonique dégagé." Hellriegel has pointed out (*Journal für praktische Chemie*, **64**, 94 [1855]) that oleaginous seeds absorb a very considerable quantity of oxygen, which, at the very commencement of germination, effects an increase in their weight.

It appeared to me that if barley could be grown in such a way as not to lose moisture by desiccation, or if a correction could be applied for loss of moisture by the grain, it would become easy to ascertain whether there is an absorption of oxygen in excess of what is necessary for the disengagement of the carbonic anhydride. The apparatus shown in Fig. 2 was found to answer admirably for this purpose; it is

merely a modified form, with certain additions, of that used in the estimation of the amount of carbonic anhydride produced.

Fig. 2.

A. Bottle containing strong potash-solution.

B. Cylinder filled with soda-lime.

C. Series of calcium chloride tubes.

This part of the apparatus is for perfectly purifying and drying the air.

D. A small mercury valve. The bend of the tube is made of a piece of capillary tubing blown on to a piece of ordinary quill-tubing, in such a way that it forms a small cup at *d*; in this cup a small globule of mercury is placed. When no air is being aspirated through the apparatus, the globule of mercury falls into the cup, and closes the orifice of the capillary tube, cutting off all communication between the two parts of the apparatus. The mercury valve is a very necessary contrivance, for without it the calcium chloride in the tubes C would absorb moisture from the tube F, and vitiate the results.

E. A copper bath, filled with water, and provided with a cover to exclude the light. The temperature of the bath is regulated in cold weather by means of a thermostat.

F. A small tube containing about 1 to 1.5 c.c. of distilled water, the use of which is to saturate the dry air with moisture before it enters the germination-flask.

G. The germination-flask; capacity about 100 c.c. To grow about 15 grams of steeped barley.

H. Chloride of calcium tube.

I. Potash-bulbs, charged with KHO solution.

K. Tube half filled with soda-lime, and half with calcium chloride.

L. Guard-tube, to which is attached the aspirator. Great care is required in fitting up the apparatus to insure its being air-tight. When connected up, a few blank experiments ought to be made to test its efficiency. Supposing it to be in good working order, then if about 3,000 c.c. or so of air are aspirated, it should be found, by weighing F, H, I, and K, before and after the experiment, that H has

gained what F has lost, and that K has gained what I has lost, within a milligram.

In cold weather the greatest care must be taken to prevent the condensation of water in the tubes from F and G. A good plan is to cover the top of the water-bath with a conical hood of brown paper, in which a current of slightly warmed air is kept circulating. The method of using the apparatus is as follows:—

A weighed quantity of steeped barley is placed in the germination-flask. Then F, G, H, I, and K, are carefully weighed. The different parts are connected together, and air is aspirated at frequent intervals for 24 hours, or as long as may be desired; at the end of that time the same parts are again weighed. The gain of I and K together give the amount of carbonic anhydride disengaged, from which may be calculated the weight of carbon oxidised; and this is, of course, the weight of carbon lost by the barley in the flask G.

The tube H in most cases gains exactly the amount of moisture which F loses, but sometimes it gains more and sometimes less. It is clear that the extra moisture must either come from, or be retained by, the flask G, and therefore a correction must be applied; in the first case, the difference in weight between H and F must be deducted from, and in the second case added to, the total loss by weight of the flask G, so that the actual loss of weight through chemical change, which is sustained by the barley, can be ascertained. By following out this plan it is found that the germinating barley seldom loses as much in weight as is represented by the carbon oxidised (as calculated from the amount of CO_2 evolved), and since we know that nitrogen is not absorbed, the cause of this difference can only be the absorption of oxygen. It follows that if the loss of weight of the barley be deducted from the weight of carbon oxidised, the residuary weight represents the quantity of oxygen absorbed and retained. The following table shows some of the results obtained:—

Days of 24 hours.	Experiment 1. Bedfordshire barley, dried at 90° F. 10·5185 grams.			Experiment 2. Bedfordshire barley, dried at 90° F. 10·4927 grams.		
	Carbon oxidised.	Loss of weight of barley.	Oxygen absorbed and retained.	Carbon oxidised.	Loss of weight of barley.	Oxygen absorbed and retained.
	gram.	gram.	gram.	gram.	gram.	gram.
1	0·0122	0·0079	0·0043	0·0062	0·0023	0·0039
2	0·0212	0·0135	0·0077	0·0207	0·0147	0·0060
3	0·0256	0·0181	0·0075	0·0266	0·0166	0·0100
4	0·0211	0·0149	0·0062	0·0229	0·0210	0·0019
5	0·0206	0·0158	0·0048	0·0196	0·0149	0·0047
6	0·0210	0·0140	0·0070	0·0226	0·0175	0·0051
7	0·0204	0·0152	0·0052	0·0240	0·0194	0·0046
8	0·0229	0·0153	0·0076	0·0222	0·0167	3·0055
9	0·0234	0·0186	0·0048	0·0231	0·0139	0·0092
10	0·0222	0·0144	0·0078	0·0239	0·0187	0·0052
11	0·0240	0·0203	0·0037	0·0243	0·0190	0·0053
	0·2346	0·1680	0·0666 = 46·4 c.c.	0·2361	0·1747	0·0614 = 42·8 c.c.

These two experiments are fair specimens of the results generally obtained. But it must be borne in mind that a fertile source of accidental error is always introduced where an apparatus, with so many caoutchouc connections, is employed; there are no fewer than 12 joints to be made as perfect as possible every time the apparatus is connected together. If (as in these experiments) the weighings are taken daily, the source of error referred to is much larger than if the weighings are taken once in every three or four days. Specimens of two experiments, conducted in the latter way, are given on page 656, and will be again referred to. It will be seen, on examining the table given above, that there is not a large preliminary absorption of oxygen before the carbonic anhydride begins to be produced; but that the oxygen required for this purpose is absorbed as it is required, in the same way as it is used by animals in respiration. It will also be seen that there is daily retained by the barley, a weight of oxygen equal to about one-third or one-fourth the weight of carbon oxidised. In some other experiments which I have made, I find that the proportion may be considerably increased, and the amount of oxygen absorbed and retained reduced almost to zero, if the air in which the barley is grown be allowed to become much contaminated with carbonic anhydride.

As to what becomes of the oxygen absorbed and retained, I hardly like to venture an opinion; I do not consider that it goes to the for-

mation of water, but rather that it is absorbed in the same way as by a saccharine wort, when, as shown by Pasteur, it combines with certain substances, and manifests its action by a deepening of the colour of the liquid. An oxidised wort of this kind, if fermented by yeast, loses the colour acquired by oxidation, probably owing to the removal of the oxygen absorbed. Now if this oxygen had formed water by its combination, it would be hardly possible for the ferment to have recovered it. Taking this into consideration, and the fact that the extra amount of oxygen absorbed by growing barley diminishes as the air in which it is grown becomes contaminated with carbonic anhydride, I am inclined to believe that this extra absorption has very little connection with the internal reaction by which the carbonic anhydride is produced.

In Experiment 1 (page 651) 0.2346 gram of carbon is oxidised to CO_2 ; to effect this, 0.5379 gram or 375.2 c.c. of oxygen are required. The oxygen absorbed and retained by the barley amounts to 46.4 c.c. The proportion between the oxygen retained and that excreted as CO_2 , is therefore $\frac{46.4}{375.2} = 1 : 8.08$. In Experiment 2 (page 651) 0.2361 gram of carbon is oxidised to CO_2 ; to effect this 0.5407 gram or 377.1 c.c. of oxygen are required. The oxygen absorbed and retained by the barley is 42.8 c.c. The ratio between the retained oxygen and that excreted as CO_2 is therefore $\frac{42.8}{377.1} = 1 : 8.81$.

Days of 24 hours.	Experiment 1. Bedfordshire barley, dried at 90° F. 10.6629 grams.			Experiment 2. Bedfordshire barley, dried at 90° F. 10.6167 grams.		
	Carbon oxidised.	Weight lost by barley.	Oxygen absorbed and retained.	Carbon oxidised.	Weight lost by barley.	Oxygen absorbed and retained.
	gram.	gram.	gram.	gram.	gram.	gram.
1	—	—	—	—	—	—
2	—	—	—	—	—	—
3	—	—	—	—	—	—
4	0.0830	0.0729	0.0101	0.0836	0.0659	0.0177
5	—	—	—	—	—	—
6	—	—	—	—	—	—
7	—	—	—	—	—	—
8	0.1023	0.0738	0.0285	0.1105	0.0763	0.0342
9	—	—	—	—	—	—
10	—	—	—	—	—	—
11	0.0680	0.0536	0.0144	0.0697	0.0510	0.0187
	0.2533	0.2003	0.0530 = 37.0 c.c.	0.2638	0.1932	0.0706 = 49.2 c.c.

I will now give the results of the two experiments (p. 656), mentioned on page 651, in both of which the same conditions of temperature and moisture were maintained as nearly as possible.

The amount of oxygen retained by the barley in the second experiment is considerably greater than that in the first. This appears to bear out the idea, stated on page 652, that there is probably no connection between the reaction by which the oxygen is retained, and that which gives rise to the carbonic anhydride. In Experiment 1, 0.2533 gram of carbon is oxidised to CO_2 . This requires 0.6754 gram or 464.1 c.c. of oxygen. The oxygen retained by the barley is 37.0 c.c. The ratio between the retained oxygen and that excreted as CO_2 is $\frac{37.0}{464.1}$ or 1 : 12.5. In Experiment 2, 0.2638 gram of carbon is oxidised to CO_2 . This requires 0.7043 gram or 491.3 c.c. of oxygen. The oxygen retained by the barley amounts to 49.2 c.c. The ratio between the retained oxygen and that excreted as CO_2 is therefore $\frac{49.2}{491.1} = 1 : 9.98$.

Many other experiments bear out the fact that there is no constant ratio between the oxygen required to produce the carbonic anhydride and that retained by the barley.

4. *The Production or Liberation of Water, by Internal Chemical Change, during Germination.*

As water is often one of the products of the complete or incomplete oxidation of organic compounds, especially where carbonic anhydride happens to be one of the bodies formed, it appeared to me to be highly probable that either a formation or a liberation of water accompanies the production of carbonic anhydride during the germination of barley. The formation of water may be illustrated by the complete combustion of a hydrocarbon, thus, $\text{CH}_4 + 2\text{O}_2 = \text{CO}_2 + 2\text{OH}_2$; and the liberation of water by the complete oxidation of a carbohydrate, thus,* $\text{CH}_2\text{O} + \text{O}_2 = \text{CO}_2 + \text{OH}_2$. The fact that a certain amount of water is formed, was suggested by Schlossberger (*Organische Chemie*, 120, 1857) as mentioned by Mulder in his *De la Bière*, p. 103: "Schlossberger, d'autre part, dit que, dans la germination, les semences ne deviennent relativement pas plus pauvres, mais qu'elles deviennent au contraire plus riches en carbone, et qu'il se dégagé, pendant la germination, une quantité bien plus grande d'hydrogène sous forme d'eau. Cette hypothèse n'est du reste appuyée de l'indication d'aucune expérience." It seems to be inferred here by Schlossberger that hydrogen is actually oxidised to form water, which hardly seems to be borne out by experiment. Later on in the same book the following passage occurs

* Empirical formula of a glucose.

(p. 120), relating to the disappearance of a portion of the carbohydrates during germination:—" Nous voyons qu'il y a eu une diminution, qui est de 82.2 à 81.0 = 1.2 : il y a donc diminution, et cette diminution est la conséquence de ce que l'on devrait désigner à present sous le nom de ' respiration de la semence pendant la germination,' et qui consiste en une absorption d'oxygène et une production simultanée d'acide carbonique et d'eau, provenant, d'une oxydation d'une partie de l'amidon qui s'était transformé d'abord en dextrine, puis en sucre." This passage seems to me to be much nearer the truth. Supposing a part of the carbohydrates to be completely oxidised, there would be liberated a certain definite amount of water for every definite quantity of carbon oxidised. Of course the ratio of the water liberated to the carbon oxidised would vary with the nature of the carbohydrate split up.

In the case of a starch, the reaction would be as follows:—

or 5 molecules of water would be liberated for every 6 atoms of carbon oxidised to CO_2 . In the case of a glucose the equation would be, $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6OH_2$, or 1 mol. of water liberated for each atom of carbon oxidised.

In the case of a saccharose the equation is $C_{12}H_{22}O_{11} + 12O_2 = 12CO_2 + 11OH_2$, or 11 mols. of water liberated for every 12 atoms of carbon oxidised.

It was found in these experiments that there is more water in the barley after germination than before. There can be no doubt that it is not *formed* directly, since the amount of oxygen absorbed by the barley, during germination, is totally inadequate for the production of so large a quantity; it must therefore be *liberated*.

The amount of water liberated, during germination, was determined indirectly, in the same way that the amount of oxygen in a carbohydrate is estimated, by determining directly the amount of carbon and hydrogen, in a known weight of the substance, and subtracting the sum of the weight of these two elements from the weight of the carbohydrate employed, to find the weight of the oxygen.

It is perfectly evident that if a certain weight of barley be germinated and dried, the weight of the dry malt (including the rootlet) plus the weight of all the known losses which barley suffers during the process, ought to be equal to the weight of the barley before germination. It is found by experiment, however, that these two weights do not balance one another. The weight of malt (together with the rootlet) plus the weight of the known losses suffered during the germinating process, always falls considerably short of the weight of the barley employed. This difference, exclusive of experimental errors, can be

due only to the liberation of water, during germination, and its subsequent volatilisation in the drying of the malt.

It must be remembered that by estimating the liberated water in this way, any error in estimating the malt produced, or the amount of the losses suffered by the barley, must fall on the number representing the water liberated. Taking this into consideration, one can hardly expect perfectly concordant results, and indeed, the nature of the experiments will hardly allow of it, however great the precautions taken may be.

The losses sustained by barley during the process of being made into perfectly dry malt, are four in number. 1. The loss due to substances removed by the steep-water. 2. The loss during the growth of the corn, which, as has been previously pointed out, is rather less than the weight of the carbon oxidised. 3. The loss due to the removal of the water originally contained in the barley. 4. The loss caused by the removal of the water liberated during germination. The loss due to volatile matter is extremely small, and may be safely neglected.

The loss during steep is estimated by carefully evaporating the steep-water, and drying at 100° C. in a weighed dish. The results obtained are very concordant. The residue is of a light brownish colour.

The loss of weight during the growth of the barley, as well as the amount of carbon oxidised, is determined in an apparatus similar to that sketched in Fig. 2 (page 649). The loss due to original water in the barley is ascertained by estimating it in a separate sample of the same barley. The method of estimating the loss due to liberated water has just been explained. The total amount of malt and rootlet produced is found by estimating the weight in a perfectly dry state.

A few words are here necessary concerning the drying of the barley and malt. The ordinary method of drying in a current of dry air at 100° C. cannot be employed without introducing two rather serious inconveniences; the first of which is that when powdered barley or malt is so treated, the oxygen of the air acts upon it in such a way that there is an easily weighable quantity of carbonic anhydride formed, but considerably more so in the case of malt, than in that of barley. The second inconvenience is, that if either of these substances be kept in a current of dry air at 100° C., they do not cease to lose weight to a very marked extent, even after the lapse of long periods (four or five days, for instance). By making use of a current of dry CO_2 , the first inconvenience is done away with, but not the last. There is, besides, a disadvantage attending the use of CO_2 , namely, that the malt, and probably also the barley, when allowed to cool in a current of this gas, occludes at least its own volume of that gas; and when a moderate quantity, say one or two grams, is being operated upon, the

amount of CO_2 absorbed easily affects the weighings. I may mention here, though it has no particular bearing on the subject, that powdered malt, when kept in the vacuum of a Sprengel's air-pump, at a temperature of about 100°C ., very slowly evolves CO_2 in small quantity for two or three days at least.

The method I finally adopted to determine the water in the barley and malt, is as follows :—

The substances were both reduced to as fine a state of division as possible, and a portion of the sample, 1.5 to 2 grams, placed in a tube (Fig. 3). The air was exhausted, and pure dry nitrogen gas admitted. The tube was gradually raised (in a salt and water bath) to a temperature of 100° to 105°C . A vacuum was again produced and maintained for five minutes, when nitrogen was introduced as before. This operation had to be repeated 32 times (the vacuum being maintained for five minutes each time) before the tube and its contents ceased to lose weight. A calcium chloride tube was placed between the tube containing the powdered barley or malt and the air-pump, in order to check the

Fig. 3.

result. The gain of the calcium chloride tube always approximated very closely to the loss suffered by the tube containing the powdered barley or malt.

I give below the results of five experiments, relating to the liberation of water during germination, which were made with the greatest care in every detail.

They were all performed on the same kind of barley, viz., Bedfordshire, harvested in 1878, and partially dried at 90°F . In each sample used, every corn was carefully selected, so as to insure similarity in all cases, as far as possible, though the conditions of temperature and moisture under which they were grown might vary somewhat.

	grams.	grams.
I. Weight of barley employed	—	10.5185
Dry malt and rootlet obtained.....	8.7558	—
Loss during steep ..	0.0685	—
Loss during actual growth	0.1680	—
Loss due to original water in barley	1.4117	—
Sum.....	10.1340	—
Difference = OH_2 liberated during growth ..	—	0.3845

Weight of carbon oxidised = 0.2346 gram.

Carbon. OH_2 liberated.

0.2346 : 0.3845 :: 12 : 19.67.

	grams.	grams.
II. Weight of barley employed	—	10·4927
Dry malt and rootlet obtained.....	8·7615	—
Loss during steep	0·0830	—
Loss during actual growth	0·1747	—
Loss due to original water in barley	1·1389	—
Sum.....	10·1581	—
Difference = OH ₂ liberated during growth ..	—	0·3346

Weight of carbon oxidised = 0·2361 gram.

Carbon. OH₂ liberated.

0·2316 : 0·3346 :: 12 : 17·01.

	grams.	grams.
III. Weight of barley employed	—	10·4361
Dry malt and rootlet obtained.....	8·6797	—
Loss during steep	0·0671	—
Loss during actual growth	0·2088	—
Loss due to original water in barley	1·1327	—
Sum.....	10·0883	—
Difference = OH ₂ liberated during growth ..	—	0·3478

Weight of carbon oxidised = 0·2417 gram.

Carbon. OH₂ liberated.

0·2417 : 0·3478 :: 12 : 17·28.

	grams.	grams.
IV. Weight of barley employed	—	10·6629
Dry malt and rootlet obtained	8·9050	—
Loss during steep	0·0612	—
Loss during actual growth	0·2003	—
Loss due to original water in barley	1·1573	—
Sum.....	10·3238	—
Difference = OH ₂ liberated during growth ..	—	0·3391

Weight of carbon oxidised = 0·2533 gram.

Carbon. OH₂ liberated.

0·2533 : 0·3391 :: 12 : 16·05.

	grams.	grams.
V. Weight of barley employed	—	10·6167
Dry malt and rootlet obtained.....	8·7491	—
Loss during steep	0·0516	—
Loss during actual growth	0·1932	—
Loss due to original water in barley	1·1523	—
Sum.....	10·1462	—
Difference = OH ₂ liberated during growth ..	—	0·4707

Weight of carbon oxidised = 0·2638 gram.

Carbon. OH₂ liberated.

0·2638 : 0·4707 :: 12 : 21·4.

The ratio between the carbon oxidised to CO_2 and the water liberated during germination, in the five foregoing experiments, is as follows :—

No. 1	12 C	:	19·67 OH_2
„ 2	12 „	:	17·01 „
„ 3	12 „	:	17·28 „
„ 4	12 „	:	16·05 „
„ 5	12 „	:	21·40 „

The mean ratio is 12 C : 18·28 OH_2 . According to this last, for every atom of carbon ($\text{C} = 12$) oxidised to CO_2 , during germination, there is liberated 1 mol. of water ($\text{OH}_2 = 18$). This agrees very closely with the equation representing the complete oxidation of a *glucose*, thus: $\text{CH}_2\text{O} + \text{O}_2 = \text{CO}_2 + \text{OH}_2$, CH_2O being the empirical formula of a glucose.