The Controlling Influence of Carbon Dioxide in the Maturation, Dormancy, and Germination of Seeds.—Part I.

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Introduction.

The cause or causes conditioning arrested development in moist seeds and the nature of the impetus which results in germination are still in most respects obscure. The problem of the non-germination of maturing seeds while still upon the parent plant and the large range of cases of delayed or non-germination of shed seeds which to all appearances are in good conditions for germination form the basis of this research.

It is to be emphasised that the problem of seed dormancy is not limited to the case of the dry seed. The more important, but less obvious, conditions of dormancy are those found in moist maturing seeds, and in cases of delayed germination in the presence of sufficient conditions of moisture and temperature. It is these which have the most interesting analogies in other fields, and an analysis of which may be more fruitful from the point of view of physiology in general.

It is useful at the outset to examine certain conclusions that are being reached by workers who have set themselves to elucidate the processes of similar phenomena in other departments of physiology. In certain aspects, the latency of the unfertilised ovum offers an analogy with the latency of moist seeds. In each case the latency is only ended by the onset of definite causes; in each case in the absence of these causes the period of latency is sooner or later terminated by death; and in each case also the sequence of changes that follow the onset of the stimulus is, in a broad sense, physiologically comparable. The interest of this analogy, moreover, is increased by the prominence which has recently been given to a simple interpretation of the nature of the fertilisation stimulus. Loeb (1) has attempted to outline its essential features as follows. These appear to be, firstly, an acceleration of oxidations which follows destruction by cytolytic agents of a cortical layer in the egg which has hitherto prevented oxygen from reaching the surface of the egg and from penetrating into the latter sufficiently rapidly. Secondly, Loeb believes that an internal change takes place which renders innocuous the toxic products of oxidation. He shows that the unfertilised matured egg dies soon, and he attributes this to the

toxic action of products of oxidation, as its life can be prolonged in the absence of oxygen.

Again, it has been a feature of recent work under many aspects to emphasise the action of the ordinary metabolic products of cell life in producing deep functional changes, both normal and abnormal. The nature of the action of these products is being studied in detail, and it has become clear in certain cases that what appears to be an act of excitatory stimulus producing a certain forward change is in reality the removal of a depressant stimulus normally present which acts as an inhibitant. Thus, for example, it has been recently shown* that the growth of the mammary glands in a pregnant female is due to a product of fœtal growth which acts by overcoming the inhibitory action of a substance which is normally present and prevents the development of these parts.

The case of antithrombin normally present in the blood in sufficient quantities to inhibit the action of any thrombin ferment formed, and so preventing any intervascular clotting, is well known. The study of immunity affords a very large number of instances of antibodies whose function is the inhibition of the harmful stimulation of poisons. Czapek (3) in his work on the anti-ferment reaction in tropistic movements of plants has added another interesting example in this line of discovery. He demonstrates geotropic stimulation to be accompanied by an accumulation of homogentisinic acid due to the action of an antiferment inhibiting its break-down by oxydase normally present.

In this paper the indicated problem of the dormancy of moist seeds has been attacked from the point of view that dormancy must be conditioned by the absence of an essential stimulus or by the presence of an inhibitory agent. The two-sided question therefore which is presented at the outset is as follows: What is the nature of the positive stimulus to germination or what is the nature of the inhibition which must be overcome to initiate this process?

Influence of Carbon Dioxide in Inhibiting the Germination of Moist Seeds.

(a) *Carbon Dioxide Inhibits the Germination of Seeds without Producing Injury.*—It will be useful to begin with a brief examination of the group of phenomena classed under the term "delayed germination." In one class of cases it is known that many seeds do not immediately germinate in nature even when to all appearance placed in optimum germinating conditions. This is true of a number of native species which remain in the ground during the winter, although freely germinating in the following spring. In another

* By Prof. Starling and Miss E. Lane-Claypon (2).

considerable class of cases the seeds appear to be capable of remaining indefinitely in the ground without germinating, while preserving latent their power of growth under certain conditions, the nature of which does not appear to be clearly understood. We find the embryos of these latent seeds to be apparently in good germinating conditions, that is, supplied with sufficient water, in an atmosphere containing the normal percentage of oxygen, and at a temperature sufficient for germination.

In a large number of cases of this phenomenon quoted by Nobbe and Hänlein (6), sporadic germination over periods of months, and even years, is a marked feature. In natural conditions *Brassica nigra* is an example of these cases of delayed germination. In Sussex it is locally called Kelke, and every farmer and labourer along the northern slope of the South Downs will give examples from his experience of the seeds sprouting in newly ploughed land after they have lain dormant for years, while the land has been under pasture or hay.

In certain of these cases of delayed germination in germinating conditions, non-germination has been shown by Ewart to be accompanied by a lack of water in the embryo due to the impermeability of the testa to water. These cases do not bear upon our problem. It is with the range of cases in which a full water supply is demonstrated that interest lies. So far as explanations based on experiment have hitherto been forthcoming for non-germination in these seeds, they have been mainly directed to elucidate this somewhat striking phenomenon from the point of view that the testa is shielding the embryo from a sufficient supply of oxygen.

Crocker (5) has reached this conclusion from his work upon the upper seeds of Xanthium burrs, which normally do not germinate till after they have lain over one year in the soil. He found that while at a temperature of 19° C. these seeds would not germinate—though containing a sufficient supply of H_2O and though lying in a normal atmosphere (*i.e.* with a partial pressure of oxygen equal to 150 mm.)—germination could nevertheless be immediately induced by removal of the testas. Recently Shull (4), working upon these same seeds, has given us the actual minimum values of oxygen necessary for the germination of the naked embryos. At a temperature of 21° C. the minimum partial oxygen pressure required by them is not more than 12 mm. If we are to adopt Crocker's view, therefore, that the non-germination of these seeds with the testa intact is due simply to the fact that only a subminimal quantity of oxygen can reach the embryo, we shall have to say that the wet testa is able to reduce the pressure of oxygen in its passage through it from 150 mm. pressure to less than 12 mm.

It is conceivable that in the maturation of the seed and in delayed

germination under ordinary and special circumstances we may be dealing not with an insufficient oxygen stimulus but with an inhibitory cause or group of causes. Such a condition might result in the case of the seed if the testa acts in any way as limiting the aëration of the embryo, for we might expect then two results:—

(1) A reduction in the amount of oxygen reaching the embryo, and
(2) A relative rise in the actual CO_2 pressure in the embryo tissues.

The crucial question first arises, therefore, as to the actual effect of increased pressures of CO_2 in the tissues of the embryo. The experiments which follow have been immediately directed to ascertain in the first place the actual effect of increased pressures of CO_2 upon the germination of quickly germinating seeds.

Technique of Experiments made to Ascertain the Effect of Increased Partial Pressures of CO_2 *on Germination.*—In setting these experiments a known quantity of pure silica sand was first introduced into large flasks and saturated with water. This was done by adding an excess of water and then drawing it off by tipping the flasks. If this was carefully done the sand was left saturated with water in a layer adhering to the bottom of the flasks. The seeds were carefully dropped on to this surface by means of a glass tube, and, where necessary, as in the case of larger seeds, a further measured quantity of H_2O was added. The flasks were then stoppered with new rubber corks fitted with one glass tube closed by means of pressure tubing and a pinchcock. Gases in any proportion desired were now quickly introduced by first withdrawing a quantity of air by an air pump, the amount being read by a pressure gauge. Where small quantities of CO_2 were desired up to 6 per cent. of an atmosphere, the operation was performed by means of a specially made apparatus on the model of Hempel's gas burette, using mercury. By means of this apparatus very accurately measured amounts of air can be withdrawn and equal lots of CO_2 introduced. For higher percentages of CO_2 the air pump was employed. The artificial atmospheres were for the most part checked by analysis after setting. The carbon dioxide, oxygen, and nitrogen employed were in all cases from cylinders as supplied by the Carbonic Acid Company and British Oxygen Company.

Table I.—The Effect of Increased Partial Pressures of CO_2 on Barley (*Hordeum vulgare*) in Retarding and Inhibiting Germination, and the Resumption of Normal Activity on Removal of these Increased Partial Pressures.

Actual percentage of CO_2 in air as set. (By analysis.)	Germinations.							Final total percentage of germination.
	In presence of raised CO_2 pressures. After			In air after removal of raised CO_2 pressures. After				
	42 hrs.	70 hrs.	118 hrs.	20 hrs.	44 hrs.	70 hrs.	Final 12 days.	
0 (air with KOH)	7	9	10					100
6·0	7	8	9					90
12·0	9	9	9					90
17·3	5	8	8					80
23·5	3	6	6	8	9	9	9	90
29·5	3	3	5	8	9	9	9	90
35·0			1	8	10	10	10	100
37·5				5	7	8	8	80
43·5				1	2	3	6	60
96·0								0

Temperature, 20° C. thermostat. 10 seeds in each experiment.

Table II.—The Effect of Increased Partial Pressures of CO_2 on Peas (*Pisum sativum*) in Retarding and Inhibiting Germinations and the Resumption of Normal Activity on the Removal of these Partial Pressures.

Approximate percentage of CO_2 in air as set. (By analysis.)	Germinations.								Final total germinations out of five seeds in each case.
	In presence of raised pressures of CO_2. After				In air after removal of raised pressures of CO_2. After				
	44 hrs.	68 hrs.	96 hrs.	7th day.	8th day.	9th day.	11th day.	20th day.	
0 (air)	1	1	5	5					5
6	0	2	3	4					4
12	1	3	4	5					5
18		1	5	5					5
24		0	1	5					5
30		2	4	4	5	5	5	5	5
50					3	4	5	5	5
70					2	4	4	4	4
100					0	1	2	3	3

Temperature, 20° C. thermostat. Five seeds in each case.

Table III.—The Effect of Increased Partial Pressures of CO_2 on Bean (*Vicia faba*), Cabbage (*Brassica oleracea*), and Onion (*Allium cepa*) Seeds in Inhibiting Germination, and the Resumption of Normal Activity on the Removal of these Increased Partial Pressures.

Species of seed used.	Percentage of CO_2 in air in which seeds were set to germinate.	Time during which seeds remain in artificial atmosphere containing raised percentage of CO_2.	Resulting germination in artificial atmosphere containing raised percentage of CO_2.	Final percentages subsequently in normal air.	
				Of germinations.	Of good plants.
		days			
Cabbage (50 seeds)	25	10	All inhibited	72	72
	38	10	,,	88	88
	44	10	,,	76	76
	0 (air with KOH)	0	Normal germination at once	84	84
Beans (30 seeds)	45	8	All inhibited	95	85
	53	8	,,	75	55
	89	8	,,	85	50
	0 (air with KOH)	0	Normal germination at once	95	85
Onion (50 seeds)	23	11	28 per cent. germinated	44	44
	30	11	All inhibited	50	50
	68·7	11	,,	46	46
	0 (air with KOH)	0	Normal germination at once	60	60

Average temperature, 17·5° C.

(b) *The Peculiar Case of White Mustard* (Brassica alba).—*Brassica alba* was peculiar among the seeds experimented on, in that inhibition was continued indefinitely after the removal of the seeds from increased partial pressures of CO_2 to normal air, and was then only terminated by the treatments described in Tables IV and V.

White mustard seeds that have been inhibited by the action of CO_2 while germinating will lie indefinitely in germinating conditions without sprouting or with sporadic sprouting over long intervals. They have all the appearance of continued vitality, and they do not become attacked by moulds. The part played by the testa in securing the continuance of the inhibitory effect of carbon dioxide after the removal of the inhibitory agent is of great interest.

In the following table it will be seen that dormancy produced by CO_2 was continued for two to three months after removal of the seeds to air, suitable conditions for germination being maintained throughout. Finally the seeds returned to normal activity and germinated 100 per cent. in every case following the treatments described.

Table IV.—The Effect of Increased Partial Pressures of Carbon Dioxide on White Mustard Seeds (*Brassica alba*) in Retarding and Inhibiting Germination, the Continued Dormancy of the Seeds after their Removal to Air and their Final Resumption of Normal Activity following Certain Definite Treatments.

Approximate percentages of carbon dioxide in air as set.	Per-centage of CO_2 by analysis after 3 days.	Germinations while in presence of raised CO_2 pressures after						Germinations in air after removal of raised CO_2 pressures.									Number of seeds which had failed to germinate at all up to this time.	Final treatment of non-germinating seeds.	Number of germinations after this final treatment.	Final total per-centage of germina-tions.
								Seeds previously in CO_2 pressures from 9 to 15 per cent. for 72 hours.					Seeds previously in CO_2 pressures above 15 per cent. for 8 weeks.							
		18	28	36	48	60	72	12	32	36	48	96	4	8	12	∞				
		hours from beginning.						hours after removal to air.					days after removal to air.							
0 (Air)	1	7	18	20	20	20	20										0			100
3	3·6	2	16	19	20	20	20										0			100
6	6·4	1	14	17	20	20	20										0			100
9	9·1		3	7	15	17	18	18	19	19	20	20*					0			100
12	12			3	11	11	12	12	14	16	18	19*					1	testas removed	1	100
15	15·5			1	2	2	2	3	7	10	14	15*					4	” ”	4	100
18	17·6						2†						4	5	13	13*	7	dried for 3 weeks	7	100
21	20·8						0†						2	5	7	9*	11	” ”	11	100
24	23·2						0†						1	1	6	6*	14	” ”	14	100

Average temperature, 17·5° C.

* Germinating conditions in air maintained for 4 weeks with no further germinations resulting.

† CO_2 pressures maintained for 8 weeks with no further germinations resulting.

Pressures of CO_2, shown in the Case of White Mustard (*Brassica alba*); and also showing the Treatments by which Immediate Germination was induced.

Description of inhibited seed used.			Time seeds lay in germinating conditions in air after removal of inhibitory partial pressures of CO_2 and the sporadic germinations that occurred.						Percentage of seeds remaining ungerminated after 90 days in air in good conditions for germination.	Treatment adopted to induce germination.	Resulting germinations when re-set to germinate after treatment.
Time during which seeds remained with inhibiting partial pressures of CO_2.	Partial pressures causing inhibition in each case.	No. of seeds.	5 days.	10 days.	20 days.	30 days.	60 days.	90 days.			
	Per cent.										
3 weeks......	24–30	40	0	0	0				100	8 seeds, testas removed ...	All germinated at once.
										4 seeds, dried	” ”
										28 continued without treatment in germinating conditions	No germination indefinitely, but not dead.
7 days	14	20	1	1	1	1			95	Remainder dried 3 weeks	All germinated in 3 days.
	24	20	0	0	0	0			100	” ”	All germinated in 3 days except two, which germinated on removal of testa.
	32	20	1	1	2	4			80	” ”	All germinated in 3 days.
	40	20	3	3	5	7			65	” ”	All germinated in 3 days except one, which germinated on removal of testa.
	52	20	7	7	9	9			55	” ”	Do. do.
16 days	20	50	0	0	0	0	7	19	62	Remainder testas removed	All germinated at once.
	50	50	0	0	0	3	18	19	62	15 dried	Most germinated at once. Those that did not, germinated on removal of testa.
										15 continued without treatment	Sporadic germination continued.
										Remainder dried 35 days	All germinated at once.
	100	50	20	41					18		
46 days	24	10	0						100	Immersed in chloroform water 70 minutes	Sporadic germination continued. 32 days later seven had germinated.
46 days	24	10	0						100	Immersed in chloroform water 40 minutes	Sporadic germination continued. 32 days later two had germinated.

From the above tables of results it is clear that the case of *Brassica alba* seeds is peculiar in that the inhibition extends after the removal of the inhibiting CO_2 pressures from the atmospheres over the seeds. This after-inhibition may extend for months accompanied by a sporadic germination. The presence and condition of the testa seem to be the controlling factors in this after-inhibition. Germination is at once induced by the removal of the testa or usually by the complete drying of the seed.

(c) *Delayed Germination in Nature.*—From these experiments it is clear that a condition is produced in the seeds of white mustard after treatment with increased partial pressures of CO_2 which very closely parallels that of seeds showing delayed germination in natural conditions. This fact is brought out more clearly when the results obtained in the laboratory with *Brassica alba* seeds are compared with the results recorded by Nobbe and Hänlein (6) as occurring in nature. These authors give a large number of cases in which they observed indefinitely delayed germination in natural conditions accompanied by the sporadic sprouting of some of the seeds extending over long periods. A few examples may be given.

Table VI.—Extract from Nobbe and Hänlein's Tables of Seeds showing Delayed Germination in Natural Conditions.

Number and name of seeds.	Germinations after —— days.								
	5	7	8	16	145	351	519	874	Finally.
Capsella bursa-pastoris, 400 seeds	3	6	—	—	10	14	34	58	75
Thlaspi arvense	—	—	1	3	—	5	15	37	87

A similar result was obtained by these authors with a large number of species. The following may be mentioned:—*Chelidonium majus, Myosurus minimus, Plantago media, Potentilla argentea, Veronica beccabunga, Chenopodium album, Campanula rotundifolia, Campanula persicifolia.*

The similarity shown in the results obtained with the seeds of *Brassica alba* inhibited under the influence of CO_2 in artificial conditions to those demonstrated by Nobbe and Hänlein (6) as occurring in natural conditions is thus very marked.

Again, Crocker (5), working on a special case of delayed germination in the upper seed of the burr of Xanthium, which, in contradistinction from the lower seed of the burr, does not germinate in the first year after ripening but in the second, found that by removing the testa he could induce immediate

germination at any time after ripening. The case of the inhibited seeds of *Brassica alba* offers an exact parallel to this case also.

(d) *Experiments Reproducing in Nature, with* CO_2 *Naturally Produced, the Results obtained in the Laboratory with* Brassica alba *Seeds.*—In drawing the foregoing parallels, a reflection which is suggested is that the inhibition of the *Brassica alba* seeds has been produced in the laboratory under conditions remote from those found in nature.

The following series of experiments were therefore directed to ascertain whether this objection is valid. The outcome of these experiments, it will be seen, is to indicate that the results of inhibition under the influence of CO_2 obtained in the laboratory with *Brassica alba* can be readily reproduced in the soil in conditions such as may occur widely in nature. The method of procedure was as follows:—Pits of various depths were dug in a garden soil consisting of sandy loam with very few stones. Short, fresh-cut grass was spread at the bottom in some cases. In others, green garden rubbish took the place of grass. The earth was then returned to the pits, and seeds, enclosed in small cotton-net bags, were inserted in it at various depths. The CO_2 content of the atmosphere of this soil at various depths was taken constantly during the experiments.

The following was a typical experiment:—On August 16, 1912, a pit 18 inches deep and 2 feet square was dug, a layer of packed green grass about 3 inches deep inserted, and the pit then filled up by the return of the earth removed. Seven days later, on August 23, three lots of 25 seeds each were buried at depths of 3, 6, and 9 inches in the earth in this pit over the grass. At the same time three control lots of seeds were placed at corresponding depths in a control pit close by, out of which the earth had been dug, and similarly returned seven days previously, but in which no grass had been placed.

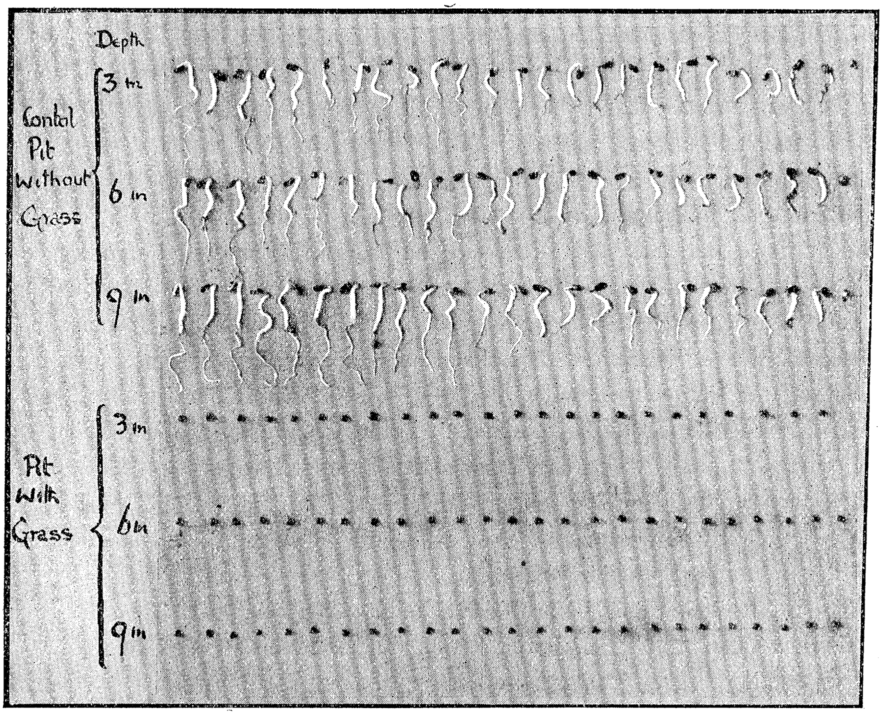
The following are examples of the percentages of CO_2 found in samples of soil air taken during the experiment at depths of 6 and 12 inches in the pit containing grass:—

	Per cent.		Per cent.
August 23 at depth of 6 inches	12·4 CO_2;	at depth of 12 inches	18·8 CO_2.
„ 29 „ „	16·5 CO_2;	„ „	20·0 CO_2.

In the earth in the control pit, without grass, the CO_2 content of the soil continued steadily at about 1 per cent. at depth of 12 inches.

After seven days in the soil the seeds were removed and examined. None of those over the pit containing decaying grass had germinated at depths of 6 and 9 inches, while at a depth of 3 inches only 3 out of the 25 seeds had sprouted. All the seeds of the control lots at each depth in the pit

without grass had germinated and sprouted vigorously. The results obtained are shown in the photograph and in the following table.



Results obtained with *Brassica alba* seeds in pit over Grass and in Control Pit without Grass after seven days.

Table VII.—Results obtained with *Brassica alba* Seeds, planted (1) in Soil over Decaying Green Grass, and (2) in Ordinary Soil.

Depths.	Germinations out of 25 seeds after 7 days.	
	Over decaying grass.	Control in ordinary soil.
inches.		
3	3 just sprouted	25 well grown.
6	0	25 ,,
9	0	25 ,,

Thus 72 out of the 75 *Brassica alba* seeds planted in soil over decaying grass were inhibited in conditions which may be supposed to occur sometimes in the soil (*e.g.*, in the ploughing in of green crops*). These seeds

* The case of heavily dunged land would also suggest itself. Boussingault and Lewy, in a large series of analyses of soil air, found 10 per cent. of CO_2 in manured soil 10 days

germinated sporadically afterwards, but systematic observations were not made in this first series of experiments as regards the after-behaviour of the inhibited seeds. A further series was, however, set in which the subsequent behaviour was noted. In this it was found that the results obtained with seeds inhibited in the soil closely conformed in all respects to the results obtained with those inhibited in laboratory conditions. In this experiment, which was conducted at a temperature of 5–7° C., and in which the seeds were left in the ground for 16 days, the CO_2 content in the soil over the buried grass rose from 10 per cent. on the 3rd day to 22 per cent. on the 16th. No germinations occurred with the *Brassica alba* seeds placed in the soil over the pit in which grass had been placed. All the seeds placed in the soil in the control pit without grass vigorously germinated within 10 days. When the inhibited seeds were removed to normal conditions of germination, 20 per cent. germinated sporadically within the first 10 days. The remainder were apparently living at the end of two months. None had been attacked by moulds. At this stage the testa was removed from a number of the seeds, with the result that germination was immediately induced, as in the laboratory experiments recorded above.

It would appear, therefore, that it is possible to reproduce in natural conditions, which may occur widely in the soil, the results obtained in the laboratory with inhibited *Brassica alba* seeds.

(e) *Action of the Testa. Bare Embryos inhibited by Carbon Dioxide.*—It is desirable now to return to the problem in its original form, in which it was indicated that germination may be due (1) to the action of a definite stimulus such as would be supplied by the access of oxygen under suitable conditions of moisture and temperature; or (2) to the removal of some inhibitory agent which has so far restrained the seed from entering upon the cycle of changes which begins with germination; or (3) to an inter-relation of both these causes.

In the experiments with carbon dioxide acting on the seed in germinating conditions so far related, it will be seen that certain partial pressures of CO_2 have the effect of retarding and inhibiting germination, the seed being capable of resuming growth without any apparent injury on the removal of the depressant. In the cases dealt with we seem to have two classes of results which must be separated. In the cases of all the seeds excepting

after treatment. It appears from these results that caution is necessary in placing seed in the ground into which green crops have been ploughed or which has been recently heavily manured. In some of the experiments with pits described above the partial pressure of CO_2 in the soil atmosphere over buried grass was found to be as much as 8 per cent. seven months after the green grass had been buried.

Brassica alba it seems clear that the CO_2 has acted directly upon the tissues of the embryo. On the removal of the CO_2 the seeds readily germinate. In the case of *Brassica alba* the action of the carbon dioxide may have been the same, but on the removal of the CO_2 from the atmosphere the seeds do not germinate but continue dormant. A direct action of CO_2 on the testa, rendering it less permeable to the passage of gases, is suggested. Such a change in the testa produced by CO_2 would have two consequences: (1) a reduction in the amount of oxygen reaching the embryo, and (2) a relative rise in the CO_2 pressure in the embryo tissues. The possibility thus arises that lack of oxygen produced by a change in the permeability of the testa due to the action of CO_2 has been the cause of inhibition in all the experiments described.

The following experiment was therefore made with *Brassica alba* seeds from which the testas had been removed.

Table VIII.—Experiment indicating that Increased Pressures of CO_2 can act Directly in producing Inhibition on the Naked Embryo of *Brassica alba*.

Percentage CO_2 in air.	Time seeds lay without germinating in presence of high partial pressures of CO_2.	Numbers and condition of seeds set in presence of high partial pressures of CO_2.	Percentage of seed germinating on removal to air.	Percentage of germinated seeds showing injury to the radicle.
	days.			
60	2	10 without testas	100	0
80	7	10 ,, ,,	100	30

The above experiment appears to demonstrate that the inhibitory action of increased partial pressures of CO_2 may be direct upon the naked embryo of mustard seeds. The phenomenon of prolonged after-inhibition did not occur in these cases in the absence of the testa. Further experiments were made with peas and white mustard with similar results. A conclusion, therefore, which appears to be justified is that, while the inhibiting effect produced on the embryo is the result of the direct action of CO_2 thereon, in the case of *Brassica alba* an accompanying change in the testa plays an important part in sealing the seed under the influence of CO_2 in a special dormant phase of life.

Summary.

Experiments were conducted showing that the germination of seeds is retarded or inhibited by high partial pressures of CO_2 in the atmosphere.

This retardation or inhibition produced by CO_2 was shown to be unaccompanied by injury. The seeds used in these experiments fall into two classes: In the first class the seeds germinated at once after removal from the inhibitory CO_2 pressures (beans, cabbage, barley, peas, onions). In the second class the inhibition continued indefinitely after the removal of the inhibitory CO_2 pressures, and was terminated only by complete drying and re-wetting, or by the removal of the testa. In this class a lowering of the degree of permeability of the testa to gases by the action of CO_2 is indicated, a change which would have two results: (1) a reduction in the amount of oxygen reaching the embryo, and (2) a relative rise in the actual CO_2 pressure in the embryo tissues. The condition of prolonged inhibition after removal to air produced in *Brassica alba* is strikingly suggestive of the condition of seeds often met with in nature, the germination of which is delayed in spite of suitable conditions of temperature and water. The results obtained in the laboratory with *Brassica alba* seeds were reproduced in the soil in natural conditions by CO_2 arising from decaying vegetable matter. The high CO_2 content of the soil air in these experiments was found to continue for a considerable period. Attention was called to the importance of these facts in agriculture.

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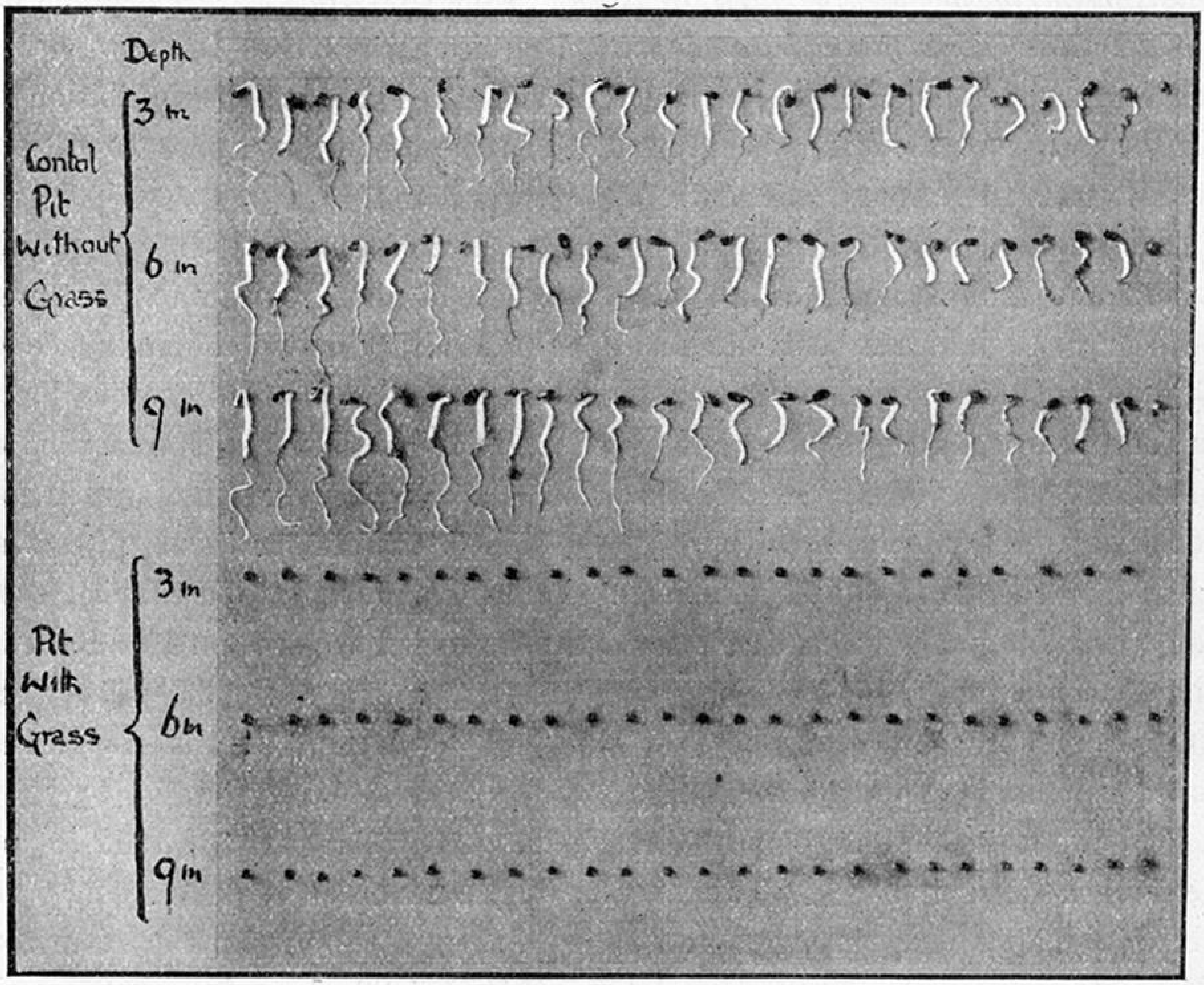

Results obtained with *Brassica alba* seeds in pit over Grass and in Control Pit without Grass after seven days.