The Ferments and Latent Life of Resting Seeds.

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This subject was suggested to me by Prof. Ewart as an outcome of his long series of experiments on the longevity of seeds.* For the most part, I have confined my attention solely to the seeds of cereals as being of the greatest importance in agriculture.†

The scheme proposed for the carrying out of these investigations necessitated the procuring of old grains; this was not an easy matter, but after several months I was very fortunate in having seeds forwarded from the Agricultural Department, Victoria; Dookie Agricultural College, Victoria; the Chamber of Commerce, South Australia; and the Hawkesbury Agricultural College, New South Wales. For these I am much indebted to Messrs. J. Knight, H. Pye, and W. Potts, whom I now take the opportunity of thanking for the trouble they have taken.

Many of the seeds obtained from the Agricultural Department of Victoria were travelling samples, and had been in the possession of the department for from 8 to 10 years, but whether they had been harvested the same season or the previous one to that in which they were put up is not exactly known. The greater number of the specimens sent from South Australia and New South Wales were accompanied by information as to the exact date of their harvesting.

The oldest grains available were samples of wheat received from South Australia which had been stored for 21 years.

The old specimens of barley, oats, and rye were amongst those previously referred to which were obtained from the Agricultural Department, Victoria, and whose minimum age must be at least 8 to 10 years. In all the above cases, certain of the different samples of grains had completely lost all their power of germination, so that they were exactly what I required in order to be enabled to carry out one section of the work. I was not quite so fortunate as regards the maize, for the oldest seeds in my possession were grown only 4½ years ago, and had only partially lost their germinating power.

* 'Proceedings of the Royal Society of Victoria,' vol. 21 (New Series), August, 1908.

† The whole of the work in the following paper has been carried out in the Botanical Laboratory of Melbourne University under Prof. Ewart's supervision, who has also critically tested and verified certain experiments and written the summary at the end of the paper. The expenses of the work were defrayed from the Research Scholarship and Apparatus Fund of the Victorian Government.

The paper is divided into sections, the first of which relates to the relative germinating powers of grains of various ages, and as far as possible obtained from different sources.

The second section deals with the connection (if any exists) between the age of the seeds and the persistence of their enzymes, with special reference to any possible co-relation between the germinating power retained by the stored grains and their enzymes.

The third section is a detailed account of experiments performed on the seeds at temperature extremes, more especially concerning their germinative capability and their enzyme reactions, as in the previous section.

The fourth section is a brief account of experiments concerning the respiratory activity of certain seeds in a more or less dried condition and the results obtained therefrom.

1. *Germinating Power of Seeds.*

The germination capacity of all the specimens received was tested, and the data so obtained are shown in the columns below. From 50 to 100 seeds from each packet (the number depending on the quantity of material at my disposal) were sown on damp blotting paper placed in glass basins, which were put under a glass frame in the conservatory to which air had free access. The temperature of the conservatory was kept fairly constant at about 23° C.

The comparative germinating capabilities are given in the following table (A):—

Germination Table A. (Wheat.)

Age.	Percentage germinated.	Place of origin.	Age.	Percentage germinated.	Place of origin.
6 months	100	S. Australia.	8½ years	32	S. Australia.
6 „	100	Victoria.	8½ „	3	Victoria.
1½ years	100	S. Australia.	9½ „	32	S. Australia.
1½ „	100	Victoria.	9½ „	0	Victoria.
2½ „	100	S. Australia.	10½ „	28	S. Australia.
2½ „	100	Victoria.	10½ „	0	Victoria.
3½ „	100	S. Australia.	11½ „	12	S. Australia.
4½ „	100	„	11½ „	0	Victoria.
4½ „	92	Victoria.	12½ „	4	S. Australia.
4½ „	90	New South Wales.	13½ „	0	„
6½ „	77	S. Australia.	15½ „	0	„
6½ „	42	Victoria.	16½ „	2	„
6½ „	39	New South Wales.	17, 18, 19,		
7½ „	68	S. Australia.	20, and 21	0	„
7½ „	16	Victoria.	years		

Germination Table B. (Barley, Oats, Maize, and Rye.)

Kind of seed.	Age.	Percentage germinated.	Place of origin.
Barley	1½ years	100	Victoria.
„	2½ „	100	„
„	4½ „	72	„
„	4½ „	54	New South Wales.
„	8½ „	18	Victoria.
„	10½ „	0	„
Oats	1½ „	96	„
„	2½ „	80	„
„	4½ „	68	„
„	5½ „	56	„
„	9½ „	0	„
Maize	6 months	100	„
„	4½ years	60	New South Wales.
Rye	6 months	100	Victoria.
„	4½ years	32	„
„	9½ „	0	„

In those cases in which different samples of Victorian seeds of the same age were experimented with, the average number which germinated is given in the tables. The majority of the seeds were sent in cotton bags, and the method in which they had been stored was not stated. In the case of wheat, however, taking for granted similar conditions of storage, the fact that the South Australian specimens retained their germination capacity for a longer period than the Victorian specimens, and the Victorian specimens retained it longer than those from New South Wales, shows that the drier the climate the longer is the life of the seed. On further reference to the wheat table, it appears that there is a well-marked drop in the germinating power of the grains after about the fourth year, and from thence it descends more or less irregularly, reaching zero in 11 to 17 years, according to the character of the sample and the conditions of storage.

2. *The Relation between the Longevity of Seeds and their Contained Ferments.*

The investigations were carried out with seeds freshly harvested and with stored seeds which had lost the faculty of germination.

The object of these investigations was to determine, as has been previously stated from time to time by different authorities, whether the loss of the germinating power was concomitant with, and caused by, or in any way related to, the disappearance of the capability of enzyme action in the seed.

The seeds used were wheat (*Triticum vulgare*), barley (*Hordeum sativum*), oats (*Avena sativa*), maize (*Zea mais*), and rye (*Secale cereale*).

In every instance, except in the maize, seeds were employed in which the

germination capacity was entirely lost, but of the oldest specimens of maize obtainable 60 per cent. of the seeds sowed germinated.

Samples of the same seeds which had been tested for loss of germinating power as described in the preceding section were first tested for the presence of *diastase ferments.*

1. *Diastase.*—The method adopted for the precipitation of the diastase was the same as that described by Darwin and Acton in the 'Physiology of Plants,' p. 305. Ten grammes of the seed were ground to a fine powder in a hand coffee mill, and placed in a bottle containing 100 c.c. of slightly warm distilled water, which was shaken for two hours with the aid of a water-motor rocker.

The mixture was then filtered and the filtrate concentrated at 50° C. under decreased pressure to about half its original volume. To the filtrate was added enough 90-per-cent. alcohol to produce a white flocculent precipitate. This precipitate was separated by filtration, and the filter-paper containing the precipitate was placed in an exhausted desiccator over sulphuric acid during the night. When dry, the precipitate was scraped off the filter-paper by means of a sterilised knife, and dissolved in a small volume of cold, boiled water.

Equal quantities of this aqueous solution were put into each of three test-tubes which had been previously sterilised, and the contents of one tube C were thoroughly boiled. A very thin starch solution was prepared, and, when cool, equal quantities of the starch solution were added to each of the test-tubes B and C and also to a fourth test-tube D. Of the four test-tubes A, B, C, D, A contained only the original aqueous solution of the precipitate. B contained about equal quantities of the aqueous solution of the precipitate and starch solution. C contained the same as B, with the exception that the aqueous solution of the precipitate had been boiled and allowed to cool before the addition of the starch solution. D contained only starch solution. The four test-tubes were placed in a bath at a constant temperature of 50° C. After about one or one and a-half hours the test-tubes were removed from the bath, and the contents of each were tested for the presence of reducing sugar by means of Fehling's test. All through the experiments only 1 drop of No. I Fehling was added to each test-tube and 2 drops of No. II. In every instance, as may be observed from the detailed lists of experiments given below, the presence of reducing sugar was detected in the contents of the test-tube B, while no sign of reduction was noted in the contents of any of the other test-tubes.

The results of these experiments, which were performed with many varied specimens obtained from widely different sources, denote conclusively the

presence of a more or less considerable quantity of a *diastase ferment* in the precipitate obtained from the extract of the crushed resting seeds. This ferment was present as such, and not as a zymogen in the resting seed, and was destroyed by boiling, as shown by the experiments performed with the test-tubes C.

The experiments were performed as far as possible in pairs, in one of which freshly harvested grain, and in the other old grain of the same kind, whose germinating power was lost, were tested.

It was impossible to carry out any additional control experiments with the aid of commercial diastase, for this was found in all cases to contain reducing sugar, and it was not found possible with the means at hand to prepare sufficient quantities of pure diastase from the material available.

It has been stated that the artificial addition of commercial diastase to ungerminable or feebly germinable seeds may bring about or increase their germination,* and the idea that ferments are connected with the vitality of seeds is a fairly prevalent one.†

In order to test this statement, samples of such seeds were sown on damp blotting paper, and a little dissolved commercial diastase was added to them. Similar numbers from the same packets of seeds were sown on damp blotting paper and, instead of dissolved diastase, a little plain water was poured over them. In addition, two similar sets of seeds were sown as above, but the seed coat of each seed, both in those treated with dissolved diastase and with water alone, was pierced once with a needle. In this way the entry of the ferment was assured even in the presence of a more or less impermeable integument.

Not all the seeds which were tested for the presence of diastase were thus treated, chiefly owing to the limited quantity of the available material, but sufficient experiments were performed with each kind of seed to ensure accuracy of results.

Effect of Addition of Diastase.—The addition of the dissolved diastase to intact seeds does not materially affect their germinating power, and in no case does it bring about germination in otherwise non-germinable seeds. The presence of external diastase aids the development of bacteria and interferes with the aeration of the seeds, which may be sufficient in some cases to

* Thompson, 'Garten-Flora,' vol. 45, p. 344, 1896; Waugh, 'Ann. Rept. Vermont Agric. Exp. St.,' 1896—7; 'Science,' N.S., vol. 6, p. 950, 1897; Sharpe, 'Mass. Hatch Exp. St.,' 1901, p. 74.

† Brnyning, Jr., F. F., "Relation entre le pouvoir germinative et l'activité diastatique de graines non-germées"; Albo, G., 'Bull. della Soc. Bot. Ital.,' 1908; 'Archiv des Sci. phys. et nat.,' vol. 25, p. 45.

produce a slight lowering of the percentage germination by preventing the germination of feebly germinable seeds.

The effect of pricking the seeds is apparently injurious, for, as a general rule, less of the pricked seeds developed than of the unpricked ones. In addition, samples of all these seeds were tested for the presence of diastase while in the resting condition, 5 to 20 grammes being used for extraction according to Darwin and Acton's method. The precipitate was allowed to act on starch solution for one hour, and then tested for reducing sugar.

Considering the case of the wheat first, as set out in the following tables, it will be observed that while age materially affects the germinating power of the seeds, it does not apparently materially influence the quantity of diastase enzyme present in the seed, nor its activity, at any rate up to an age of 20 years.*

In every instance, strong reduction was produced by Fehling's test, and the variations in the degree of reduction noted in the tables were extremely slight, and might be due to differences between the samples when originally harvested.

The different samples of barley gave closely similar results, and the same applies to the oats, rye, and maize.

The oats exhibited the greatest amount of variation, and though in all cases a distinct reduction was obtained on testing for diastase, the amount present in some of the resting seeds was small. Slight differences in the amount of reduction are in part produced by extraneous factors, such as the strength and quantity of the solutions used, the fineness to which the seeds are ground, and the detailed treatment during extraction. These results do not coincide with those obtained by Acton,† who found that an extract from wheat grains which had been stacked for 28 years exercised no diastatic action on thin starch solution. He offers the suggestion that the diastase present in the freshly stored grains had been destroyed by oxidation, or by the influence of micro-organisms. Thus reference to the two first cases of oats cited in the tables shows that 10 grammes of fresh oats, with a germination capacity of 100 per cent., produced exactly the same reduction on testing as was produced by 10 grammes of oats from 8 to 10 years old, with a germination capacity of *nil*, under as precisely similar conditions as possible.

The least reduction was produced by maize, the strength of reduction in

* Brocq-Rousseau and Gain ('Compt. Rend. Acad. Sci. Paris,' vol. 146, 1908, p. 545) state that peroxidase enzymes appear in seeds up to 20 years of age and may persist in some cases for 100 to 200 years.

† 'Annals of Botany,' vol. 7, No. 27, September, 1893.

Diastase Enzymes.

Kind of seed.	Age.	Reduction with Fehling's test.	Percentage of seeds germinated.	Percentage of seeds germinated with diastase.	Place of origin.
Wheat	21 years	Fairly strong	0	—	S. Australia.
,, ...	20 ,,	Strong	0	—	,,
,, ...	19 ,,	Fairly strong	0	0 (unpricked) 0 pricked	,,
,, ...	18 ,,	Very strong	0	—	,,
,, ...	17 ,,	,,	0	—	,,
,, ...	16 ,,	Strong	0	—	,,
,, ...	5 months	,,	100	—	Victoria.

Diastase Enzymes.

Kind of seed.	Age.	Reduction with Fehling's test.	Percentage of seeds germinated.	Percentage of seeds germinated with diastase.	Place of origin.
Wheat	13 years	Strong	0	—	S. Australia.
,, ...	12 ,,	,,	2	—	,,
,, ...	11 ,,	,,	12	—	,,
,, ...	10 ,,	,,	28	24 (unpricked) 20 (pricked)	,,
,, ...	9 ,,	,,	32	28 (unpricked)	,,
,, ...	8 ,,	,,	32	25 ,,	,,
,, ...	7 ,,	,,	62	—	,,
Wheat (Steinwedel)	8 to 10 years	,,	0	—	Victoria.
,, (Duleith) ...	11½ years	,,	0	—	,,
Wheat (Indian King)	6 ,,	,,	4	0 (unpricked)	,,
Barley	5 months	,,	100	—	,,
,, (Algerian)	8 to 10 years	,,	0	0 (pricked) 0 (unpricked)	,,
,, (Chevalier)	8 to 10 years	Slight	0	—	,,
Barley (Hallet Chevalier)	4 years	Very strong	54	51 (unpricked)	New South Wales.
Oats	1½ ,,	Slight	100	80 ,,	Victoria.
,, (Golden)	8 to 10 years	,,	0	0 (pricked) 0 (unpricked)	,,
,, (Algerian) ...	5 years	,,	56	56 (unpricked)	,,
,, ...	3 ,,	Fairly strong	84	—	,,
,, ...	2 ,,	,,	96	—	,,
,, ...	3 ,,	,,	60	—	,,
,, (Calcutta) ...	4½ ,,	Slight	72	—	,,
,, ...	4½ ,,	Fairly strong	76	—	,,
Rye	6 months	Strong	100	—	,,
,, ...	4½ years	,,	32	32	,,
,, ...	8 to 10 years	,,	0	—	,,
Maize	6 months	Slight	100	—	,,
Maize (Golden King)	4½ years	,,	60	52	New South Wales.

the two specimens used being the same; but as material which had entirely lost its power of germination was not obtainable, the results in this case are not perhaps quite so convincing as in the case of the other cereals.

It might be urged that since the resting seeds were not stored in an absolutely dry condition, the persistence of the diastase might be due to its being reproduced as fast as it decomposed, but a comparison of the South Australian results with those from Victoria and New South Wales does not give any evidence of this.

2. *Proteolytic Enzymes.*—The first method adopted for the demonstration of these ferments was approximately the same as had been employed by Prof. Vines in his paper on "The Proteases of Plants,"* the difference being that in this case from none of the seeds had the integuments been removed before grinding.

The grain, 10 grammes in each case, was crushed by the hand mill, and put into 100 c.c. of distilled water and shaken for two hours. The material was then filtered and the filtrate was used as the digestive solution without precipitation of the enzymes.

Into bottles containing 50 c.c. of this solution were put 3 drops of hydrocyanic acid and 0·2 gramme of well-washed fibrin which had been carefully preserved in spirit. Into a similar bottle were put 50 c.c. of the same solution which was thoroughly boiled, and when cold 0·2 gramme of washed fibrin and 3 drops of HCN were added to this bottle also. These bottles were put into the oven at a temperature of 36° C. As in the case of diastase, control experiments in which commercial pepsin was employed were rendered impossible owing to the constant presence of traces of peptone mixed up with the pepsin, which was obtained both in the form of a powder, and as scales, but always containing the same impurities. Attempts to separate the pepsin and peptone by fractional dialysis failed.

After about 20 hours in the oven, application of the biuret test showed the presence of slight traces of peptone in the unboiled specimens, whilst the boiled specimens when similarly tested only gave the violet colour characteristic of undigested proteids.

On treating some of the aqueous solution from the seeds in exactly the same way as the above, with the single exception that no fibrin was added to the bottle, application of the biuret test showed the presence of minute quantities of peptone, thus indicating the occurrence of autolysis. The results obtained in this way are tabulated along with those obtained when dealing with solutions prepared by another method to be now described. In order to diminish autolysis and to obtain more satisfactory results, the

* 'Annals of Botany,' vol. 20, 1906, p. 115.

enzymes were precipitated from their solutions. The method followed was almost identical with that described by Dean* when dealing with the proteolytic enzymes of *Cucurbita pepo*. Twenty grammes of the grain were ground and mixed with 100 c.c. of cold, boiled water, and shaken for two hours. The mixture was then filtered and the filtrate precipitated by a volume of saturated ammonium sulphate equal to that of the original filtrate. A white, more or less flocculent precipitate was thrown down, which was filtered off and dried overnight in an exhausted desiccator over strong H_2SO_4.

The precipitate was scraped off by means of a sterilised knife as before, and dissolved in a small amount of cold, boiled water.

In testing for fibrin-digesting enzymes, 1 c.c. of the aqueous solution produced above was put into each of three sterilised test-tubes A, B, and C. The contents of the test-tube C were boiled well and allowed to cool. Into B and C were put 0·2 gramme of well-washed fibrin, and 5 drops of chloroform were added to each of the three test-tubes. The three test-tubes provided with corks were placed in the oven at 35° C. and left for an interval of time varying from two to four days.

Examination of the contents of all these test-tubes for the detection of peptone showed that B and C both gave faint biuret reaction, the boiled as well as the unboiled specimens.

A little of the liquid from each test-tube was examined under the microscope, with the result that it was found to be swarming with bacilli, by which the conversion of proteids into peptones had been wholly or partially effected.

This difficulty regarding the bacteria was due to the prolonged time necessary for the action of these ferments to become manifest; and it was evident that the addition of 5 drops of chloroform, and the subsequent plugging of the test-tubes, was inadequate to maintain antisepsis, so that it was necessary to adopt more stringent measures to destroy the bacteria, but still not to impede in any way the action of any digestive ferments which might be extracted from the resting seeds.

After many unsuccessful attempts to find some means of effectively fulfilling these conditions, the required end was attained by soaking the grain in strong chloroform for about five minutes after weighing it, and grinding it in the mill while still wet. Care was taken that all the bottles, apparatus, and water employed throughout the experiments were thoroughly sterilised beforehand. Prior to putting the test-tubes into the oven 5 drops of chloroform were added, and the tubes were stopped with plugs of cotton wool. In this way complete sterility was produced.

* 'Botanical Gazette,' vol. 39, May, 1905, "Proteolytic Enzymes," p. 331.

The detailed results are enumerated in tabular form below, from which it will be seen that a proteid digesting enzyme is present, which is destroyed by moist heat at 100° C. As in the case of diastase, germination tests were performed in order to discover whether the germinating power was affected by the addition of commercial pepsin solution, or bore any relation to the persistence of the proteolytic enzymes in the resting seeds. Throughout the experiments the temperature of the oven was between 34° C. and 40° C.

Fibrin-digesting Proteolytic Ferments.

Kind of seed.	Age.	Method.	Time of digestion.	Reaction with biuret test.	Percentage germination.	Percentage germination with pepsin.	Place of origin.
Wheat	6 months	V.	50 hours	Faint	100	100	Victoria.
,, ...	21 years	V.	60 ,,	,,	0	—	S. Australia.
,, ...	20 ,,	V.	60 ,,	,,	0	—	,,
,, ...	6 ,,	D.	50 ,,	,,	0	—	,,
,, (Indian King)	6 ,,	D.	48 ,,	,,	8	4	Victoria.
Wheat	2½ ,,	D.	40 ,,	,,	68	68	S. Australia.
Barley	6 months	V.	60 ,,	,,	100	100	Victoria.
,, (Algerian)	8 to 10 years	V.	60 ,,	,,	0	0	,,
,, (Golden Drop)	8 to 10 years	V.	60 ,,	Very faint	14	8	,,
Barley (Hallet Chevalier)	4½ years	D.	48 ,,	Faint	54	25	New South Wales.
Barley	4½ ,,	D.	60 ,,	,,	100	—	Victoria.
Oats	5½ ,,	V.	60 ,,	Very faint	56	40	,,
,, (Golden).....	8 to 10 years	V.	60 ,,	,,	0	0	,,
,, ...	1½ years	V.	60 ,,	,,	92	81	,,
,, ...	2½ ,,	D.	60 ,,	Faint	96	92	,,
,, ...	4½ ,,	D.	60 ,,	,,	62	58	,,
Rye	6 months	D.	64 ,,	Fairly good	100	96	,,
,, ...	4½ years	D.	64 ,,	,,	32	29	,,
,, ...	8 to 10 years	D. & V.	64 ,,	,,	0	0	,,
Maize	6 months	V.	70 ,,	Slight traces	100	—	,,
,, (Golden King)	4½ years	D. & V.	70 ,,	,,	60	60	New South Wales.

V. = Vine's method of preparing the proteolytic ferments, 'Annals of Botany,' No. 78, April, 1906, p. 115.
D. = Dean's method, 'Botanical Gazette,' vol. 39, May, 1905, p. 331.

Reference to the preceding tables shows that a fibrin-digesting enzyme is present in minute quantity in the resting grains of the cereals investigated, and that apparently its amount is not appreciably influenced by the age of the grains. Judging the amount of the ferment by its activity the amount present is small in all the seeds tested, the maximum activity being possessed by the rye seeds, and the minimum by the maize. The addition of commercial pepsin solution to the seeds does not in any case increase their percentage germination, and where the percentage germination is low tends to lower it still further.

Test for Erepsin.—Further investigations were carried on in order to find out whether erepsin ferments were present in the resting grains. The mode of precipitation of the enzyme has been previously described, the same method being employed as was used in testing for pepsin. Before grinding, the seeds were soaked for about five minutes in strong chloroform, as was done when testing for the existence of fibrin-digesting ferments in the seeds. One cubic centimetre of the aqueous solution of the precipitate produced by the saturated ammonium sulphate was put into each of three sterilised test-tubes A, B, and C. The same difficulties arose in the case of the samples of prepared pancreatin as were met with in the diastase and pepsin, the samples purchased giving the tryptophane reaction owing to the presence of amide impurities. As in the case of the fibrin-digesting enzyme, the contents of the test-tube C were boiled and allowed to cool. About 0·2 c.c. of Witte peptone was added to each of the tubes B and C; 5 drops of chloroform were dropped into each of the three test-tubes, and the mouths of the tubes were plugged with cotton wool. The three test-tubes were placed in the oven, which was kept at a temperature of 35° to 36° C. throughout the series of experiments. The test-tubes were left in the oven for about three days, during which time they were occasionally shaken at intervals.

The test adopted for the detection of products of erepsin digestion such as amides was the tryptophane test. About 4 or 5 drops of bromine water were added to each of the contents of the test-tubes A, B, and C, and without exception tryptophane was produced in the contents of the tube B, whilst no trace was observed in A or C.

The results demonstrate the occurrence of an erepsin ferment in the seeds, which is destroyed in water at 100° C. Experiments were also performed to determine the percentage of seeds capable of germination under the action of a solution of commercial pancreatin in water. The results of these experiments are stated in a special column of the tables below.

As in every instance the tryptophane reaction was well marked, in order to economise material the experiments for the detection of erepsin were limited to one sample of fresh and one of old grains.

The quantity of erepsin present must be fairly considerable as judged by the degree of activity of the extract.

Reference to the tables shows that no favourable effect on the germinative capacity of the seeds is noticeable as the result of soaking in weak pancreatin solution. The addition of a weak solution of pancreatin did not favour germination, but rather the reverse, except in the case of 4½-year-old rye. On repeating this latter test, however, the seeds only gave

Erepsin Ferments.

Kind of seeds.	Age.	Method.	Time acting on peptone.	Trypto-phane reaction.	Per-centage germina-tion.	Percentage germination with pancreatin.	Place of origin.
Wheat	2½ years	D.	65 hours	Good	100	98	S. Australia.
„ (Marshall's Prolific)	8 to 10 years	D.	65 „	„	14	11	Victoria.
Barley (English)...	4½ years	D.	65 „	„	44	44	„
„ (Algerian)	8 to 10 years	D.	65 „	„	0	0	„
Oats	1½ years	D.	65 „	„	92	73	„
„ (Golden)	8 to 10 years	D.	65 „	„	0	0	„
Rye	6 months	D.	65 „	„	100	—	„
„	8 to 10 years	D.	65 „	„	0	0	„
„	4½ years	D.	64 „	„	34	30	„
Maize	6 months	D.	65 „	Fairly good	100	—	„
„ (Golden King)	4½ years	D.	65 „	„	60	60	New South Wales.

D. = Dean's method of extracting erepsin, 'Botanical Gazette,' vol. 39, May, 1905, p. 331.

30 per cent. germination after treatment with pancreatin, so that the apparent rise was of accidental origin.

In concluding this section of the paper, the net results may be briefly summarised as follows :—

1. Although the germinating power continually decreases with advancing age, the enzymes persist comparatively unaffected.

2. Diastase is present in fairly large quantities in both fresh and old resting seeds of wheat, barley, oats, rye, and maize, being least active in the latter.

3. A fibrin-digesting ferment is present in traces in all the above-mentioned seeds.

4. Erepsin is present in considerable amount in all the above-mentioned seeds.

5. All these ferments are destroyed by being raised to the temperature of boiling water in the presence of moisture, but they are not destroyed by the immersion of the seeds for about five minutes in strong chloroform.

6. The maximum quantity of all the enzymes occurs in rye, and the minimum quantity in the maize.

7. The addition of dissolved ferments does not increase the percentage germination of old seeds, and where any effect at all is produced tends to lower it.

The Effect of Extremes of Temperature on the Germinating Power and Enzyme Contents of Seeds.

High Temperatures.—All the enzymes present in the resting grains are completely destroyed by moist heat at 100° C., and the same is true of the germinating power of the grains, no signs of germination being apparent in the seeds of cereals which have been immersed in boiling water for a few minutes. This appeared to suggest the possibility of some co-relation existing between the germinating power and enzyme reaction of seeds regarding their powers of resisting high temperatures, irrespective of the fact that no such co-relation exists between these two phenomena as regards their capacity for withstanding time.

To test whether any such connection really exists, many experiments were carried out, using dry instead of moist heat.

It is important that the seeds should be as nearly completely dry as possible, and for this reason before being used for an experiment they were taken from the store room, which was the driest place obtainable, and placed in sulphuric acid desiccators kept in an oven at about 35° C. for a week or more. The first series of experiments was carried out at 100° C., and the method adopted was as follows:—The dried grains were placed in perfectly dry test-tubes, which were fitted into holes in a sheet of cardboard. The test-tubes containing the seeds were placed in a vessel containing boiling salt solution, and the bulb of a thermometer was put into one of the test-tubes among the seeds. The sheet of cardboard prevented any steam reaching the open ends of the test-tubes, so that the grains were kept perfectly dry. The grains were kept at a temperature of 99° to 100° C., for different intervals of time varying from $\frac{1}{4}$ hour to 16 hours, and on removal from the test-tubes their germination capacity and enzyme reactions were investigated. The results of these investigations may be obtained in detail from the following tables.

The germinating power became gradually weakened as the interval of time during which the grains were subjected to this high temperature increased, but the same did not apply to the enzymic activity, for after 16 hours' exposure to 99° or 100° C. the actions of the enzymes were apparently in no wise impaired.

A different method had to be adopted in order to raise the temperature of the grains above 100° C. This was done by spreading the seeds, which had been previously dried in the desiccator, as before in a single layer, and placing them in an oven heated to the required temperature. The temperature of the oven was first raised to 120° C. and kept constant for an hour. The

seeds thus treated were afterwards tested for their germinative capacity and their enzyme reactions, the former of which was found to be entirely lost in each kind of seed used, while the latter was still evident, though in certain cases it was markedly diminished.

The highest temperature at which the slightest possible traces of enzyme reactions remained visible was 130° C., when faint signs of saccharification of starch were produced by the diastase extracted from resting grains of barley subjected to this temperature. Throughout this series of experiments only fresh seeds were employed, whose germinating capacity before exposure to the high temperatures was approximately 100 per cent.

After the grains had been heated above 120° C. the nature of the precipitates was apparently changed. While the bulk of the precipitate was seemingly as copious or even more so than before the heating of the seeds, it was much more soluble in water than that obtained from the same material unheated, and the filtrate was thinner and less glutinous than before. This was especially pronounced in the rye, for the filtering process in this case lasted about two hours, while the same process with the fresh seeds which had not been exposed to high temperatures occupied as many days.

The tables appended below show the results of these experiments in detail, *i.e.* the effects produced in the seeds on exposure to abnormally high temperatures; the effects of extremely low temperatures on the seeds will be dealt with later.

The methods of precipitating both the diastase and the proteolytic enzymes were the same as those employed in the preceding section of the paper.

Briefly summarising the results set down in the tables, it is found that the most resistant of all the ferments to extremes of heat is the diastase of barley, which is not absolutely destroyed till the grains have been heated to 131° C. for an hour. The least resistant of the enzymes is apparently the fibrin-digesting enzyme, for it is destroyed entirely at 124° C. in every kind of seed tried. This result may, however, possibly be connected with the fact that the quantity of this ferment present even in the fresh grains is extremely small.

Whether the slight variation in the resistant power of the diastatic and proteolytic enzymes of different grains to dry heat indicates the existence of specific varieties of the different enzymes must remain for the present an open question, but in any case the most exact experiments merely indicate that the ferments in question are no longer capable of extraction and do not say whether they have been actually destroyed or merely coagulated and rendered insoluble. The coagulation temperature in the different seeds might

Temperature Extremes. High Temperatures.

Kind of seed.	Temperature.	Time seeds exposed.	Percentage germination.	Reduction with Fehling's test.	Biuret reaction.	Tryptophane reaction.
	° C.	hours.				
Wheat*	99—100	½	48	Strong	Faint	Good.
Barley	99—100	½	32	,,	,,	,,
Oats	99—100	½	48	,,	,,	,,
Wheat.....	99—100	1	24	,,	,,	,,
,, ...	99—100	4½	0	,,	,,	,,
Barley	99—100	4½	6	,,	,,	,,
Oats	99—100	4½	24	,,	,,	,,
Barley	99—100	6½	0	,,	,,	,,
Rye	99—100	6½	0	,,	,,	,,
Maize	99—100	6½	0	,,	,,	,,
Wheat.....	99—100	16	0	,,	,,	,,
Barley	99—100	16	0	,,	,,	,,
Oats.....	99—100	16	0	,,	,,	,,
Wheat.....	122	1	0	,,	Very faint	Fairly good.
Rye	122	1	0	Faint	,,	Good.
Maize	122	1	0	Faintest trace	,,	Very faint.
Wheat.....	124	1	0	Strong	None	Fairly good.
Rye	124	1	0	Faintest trace	,,	Faintest trace.
Maize	124	1	0	None	,,	None.
Oats.....	126	1	0	Faintest trace	,,	,,
Wheat.....	127	1	0	Faint	,,	Faint.
Oats.....	127	1	0	None	,,	None.
Wheat.....	128	1	0	Faintest trace	,,	Faintest trace.
,, ...	130	1	0	None	,,	None.
Barley	130	1	0	Faintest trace	,,	,,
Oats.....	130	1	0	None	,,	,,
Barley	130	1	0	,,	,,	,,

* [In my paper on the vitality of seeds, wheat and barley are given as withstanding a day's dry heat at 100° C. The error is due to the transcription of 1 h. into 1 d., and the records are for one hour's heating and not one day's, the somewhat higher percentages being possibly due to more perfect drying.—Alfred J. Ewart.]

naturally vary somewhat, since their structure, composition, and power of retaining moisture all vary to a certain extent.

The diastase and the erepsin of the resting seeds appear to be almost equally resistant to dry heat, or at least there is more variation between the diastases of different resting seeds than between the diastase and erepsin of the same seed.

Above 100° C. no seeds of any kind were found to be capable of germination, and the germinating power was absolutely lost in those seeds which had been subjected in a dry condition to a temperature of 99° to 100° C. for 5½ hours. Just* showed that as seeds are dried their resistance to dry heat increases, and von Hohnel† found that many fully dried seeds could withstand an hour's exposure at 110° C.

* 'Cohn's Beiträge,' vol. 2, 1877.

† 'Haberlandt's Wiss.-prakt. Unters.,' vol. 2, 1877.

Low Temperatures.—The exposure of the grains to low temperatures, both in the dry and moist conditions, had different effects from their exposure to high temperatures. The mode of carrying out these investigations was as follows: Seeds of fresh wheat, barley, oats, rye, and maize were dried in the same manner as when testing for the effects produced by abnormally high temperatures, and placed in perfectly dry glass tubes, which were carefully sealed off, but which previously to sealing had been weighted with shot.

Samples of the same kinds of seeds were put together with shot into loosely woven muslin bags, and the tubes and bags were lowered into a flask of liquid air. The weighting of the tubes and bags was necessary owing to the specific gravity of the liquid air being about equal to that of water.

The liquid air remained in the flask for about three days and all the seeds were completely immersed in it for fully two days.

The seeds were removed from the liquid air and some of each kind were set for germination, while corresponding seeds from the same packets which had not been subjected to the temperature of the liquid air were also set to serve as controls. Also some of the seeds from the tubes and muslin bags were ground up and their ferments precipitated as before. Neither the germinating power nor the enzyme reactions appeared to be appreciably affected in the case of any of the cereals by the exposure to the extreme cold of the liquid air, the temperature of which is approximately $-200°$ C. No constant difference was noticeable between the effects of exposure in sealed tubes and of exposure in muslin bags where the seeds were in direct contact with the liquid air.

The slight drop in the percentage germination after exposure to liquid air in sealed tubes in the case of barley and rye, and in the case of wheat, oats, and rye where the seeds were in direct contact with the liquid air, is probably the result of these samples containing a few seeds whose power of germination was at a low ebb. In any case the differences are very small, and would be almost within the limit of error, were they not all on the same side.

As no means were available of obtaining lower temperatures, it was impossible to arrive at the satisfaction of destroying the ferments by abnormally low temperatures, if this be possible. Somewhat similar sets of experiments were performed by Brown and Escombe,* who kept various kinds of seeds exposed to liquid air enclosed in vacuum-jacketed tubes for 110 hours, and then slowly thawed them. They proceeded to test the germinating power of these seeds together with control specimens, but did not discover any appreciable difference between that of the seeds which had

* 'Science,' N. Ser., vol. 8, 1898, p. 215.

been exposed to liquid air and the control specimens which had not been so exposed. The same conclusions were arrived at by Thiselton-Dyer,* who subjected seeds to a temperature of −250° C. for a shorter period. Becquerel† also performed experiments dealing with this subject.

The results of the experiments performed are as follows :—

Temperature Extremes. Low Temperatures.

Kind of seed.	Percentage germination (normal).	Percentage germination after liquid air.	Reduction with Fehling's test.	Biuret reaction after fibrin digestion.	Tryptophane reaction after Witte-peptone digestion.	Contained in—
Wheat	100	100	Strong	Faint	Good	Sealed tube.
Barley	100	96	,,	,,	,,	,,
Oats	92	92	,,	,,	,,	,,
Rye	96	90	,,	,,	,,	,,
Maize	90	90	,,	,,	,,	,,
Wheat	100	96	,,	,,	,,	Muslin bag.
Barley	100	100	,,	,,	,,	,,
Oats	92	90	,,	,,	,,	,,
Rye	96	92	,,	,,	,,	,,
Maize	90	90	,,	,,	,,	,,

As regards the ferments, there was not the faintest perceptible difference between those precipitated from the two sets of seeds, although it must be remembered that a difference will only be perceptible when a relatively large part of the original amount of ferment has been destroyed or rendered inactive or insoluble.

It is of great interest to note that the enzymes present within the resting grains of the five different genera of cereals employed throughout these experiments are not destroyed when the thoroughly dried seeds are subjected to the extraordinarily wide range of temperature of −200° C. to +120° C., *i.e.* a range of 320° C.

The enzymes of a few varieties of seeds such as the diastatic ferment of barley retains a certain amount of its activity when the range of temperature through which the seeds have been exposed is −200° C. to 130° C., *i.e.* a range of 330° C.

The range of temperature through which the capacity for germination is retained is from −200° C. to 100° C., *i.e.* a range of 300° C., above this the power is apparently entirely lost.

The conclusions arrived at in this section serve to substantially verify that drawn from the last section, that the capacity for germination is not

* 'Roy. Soc. Proc.,' vol. 65, p. 362, 1899.

† 'Ann. Sci. Nat., Bot.,' ser. 9, vol. 5, 1907.

dependent upon the existence of enzymes in the resting seeds of the cereals mentioned, although the question will not be absolutely closed until it is found possible to germinate seeds which contain no enzymes in the resting condition, or in which these enzymes have been destroyed. Since enzymes appear to retain their activity within a wider range of conditions than does the capacity for germination, this is likely to be a matter of the utmost difficulty, or may be impossible.

Before concluding the series of experiments in connection with this section of the paper the resistance to extreme cold of certain other varieties of seeds was also tested. Some of the different kinds of seeds were tied up in loosely woven muslin bags, together with shot to ensure their sinking when immersed in liquid air. The bags were lowered into a flask of liquid air in which they were left for one and a half days.

One hundred of each kind of seeds from the liquid air were set to germinate on damp blotting paper in a special germinating box, whilst 100 of each kind which had not been exposed to the extreme temperature of liquid air were set to germinate on damp blotting paper alongside them. The varieties of seeds employed were chosen from sorts possessing widely differing characters, including some which, being sensitive to desiccation, might also be sensitive to extreme cold.

The names of the seeds used are enumerated in the tables, accompanied by the relative numbers which germinated under normal conditions and after exposure to liquid air respectively. The third column contains data supplied by Prof. Ewart for comparison between the resistance to extreme cold and to desiccation.

Reference to the table shows that in not a single instance were the seeds entirely killed as the effect of their immersion in liquid air.

The influence of the low temperature is naturally most pronounced in the case of samples with a comparatively low germination capacity in which a number of the seeds are only just able to germinate under the most favourable conditions.

In the case of the carrot seed, freezing appeared to increase the percentage germination, but on re-testing the original seeds a percentage germination of 65 was obtained; possibly the first test was discontinued too soon.

The liquid air apparently exerts a retarding influence on the germination as, except in the isolated case of the cress seeds, in which signs of germination were apparent in 100 per cent. of both sets of seeds one day after sowing, germination was always noticeable in the seeds grown under ordinary conditions before those which had been subjected to the intense cold of liquid air.

Germination Tables.

Kind of seed.	Percentage germination. (Normal.)	Liquid air.	Resistance to desiccation.
Apple (*Pyrus malus*)	12	4	Sensitive to severe desiccation.
Turnip (*Brassica campestris*)	91	88	43 per cent. after 42 days' desiccation at 37° C.
Cress (*Lepidium sativum*) ...	100	100	30 per cent. after 4 weeks in absolute alcohol.
Carrot (*Daucus carota*)	36 to 65	59	Lasts 10 years in dry air.
Haricot (*Phaseolus multiflorus*)	100	90	2 per cent. after 45 days' desiccation at 37° C.
Hemp (*Cannabis sativa*).....	24	7	Nil after 15 days' desiccation at 37° C.
Mustard (white) (*Brassica alba*)	85	72	Lasts 10 years in dry air.
Lobelia erinus	51	13	Sensitive to prolonged extreme cold (De Candolle).
Parsnips (*Peucedanum sativum*)	45	18	Sensitive to extreme desiccation.
Parsley	28	1	„ „ „
Pea (*Pisum sativum*)	95	75	Nil after 42 days' desiccation at 37° C.
Radish (*Raphanus sativus*)...	97	88	Lasts 10 years in dry air.
Ricinus cambogiensis	100	100	*R. communis* 40 per cent. after 28 days' desiccation.
Sunflower (*Helianthus annuus*)	70	65	51 per cent. after 42 days' desiccation at 37° C.

The fact that in every experiment except two there is a lower percentage germination in the severely frozen seeds, and that in no instance is the reverse the case, signifies that to some extent freezing in the liquid air is deleterious to the germinative power of seed. Another noteworthy observation is that there does not appear to be any particular class of seed which is more injured by the extreme cold than any other class, *e.g.*, of three kinds of oily seeds tested, viz., Hemp, Helianthus, and Ricinus, while the first was strongly affected, the second was little, and the last-named seed not at all injured by −200° C. for two days.

The starchy seeds of cereals are, however, as resistant to the effects of exposure to liquid air as are the oily seeds.

Lobelia erinus seeds were selected as good subjects for experiment on the strength of the statement of De Candolle* that dry seeds of *Lobelia erinus* lose their vitality sooner at very low temperatures than at ordinary ones.

The results tabulated in this paper show that the vitality of some is lost, but as 13 per cent. were found to germinate after exposure for one and a half to two days to a temperature of approximately −200° C., it is probable that if the time of their exposure were increased somewhat the vitality of all the seeds would be destroyed.

* Pfeffer, 'Physiology of Plants,' Engl. translation, vol. 2, p. 234.

From the third column on the list it can be seen that, on the whole, though not without exception, the resistances to extreme cold and to extreme desiccation are approximately parallel.

4. *The Respiratory Activity of Resting Seeds.*

In this series of experiments the respiration of certain other characteristic kinds of seeds was tested in addition to the foregoing cereals.

Whether dried seeds respire at all, and if they do to what extent, is one of the most discussed problems in plant physiology, especially in connection with the views as to whether the life in resting seeds is merely at a low ebb or is entirely suppressed.

The apparatus employed was Aubert's improved form of that of Bonnier and Mangin, and, as was stated in a previous paper dealing with the respiration of gynæcia,* the machine gave complete satisfaction, provided that certain precautions were taken. Before each set of experiments the mercury was removed from the apparatus and was thoroughly cleansed by several washings in strong hydrochloric acid, followed by several washings in distilled water, and then being passed through a filter to dry.

This precaution was found to be of extreme importance, for in the presence of any impurities such as zinc in the mercury, the inlet of a sample of air into the tube produced oxidation of the zinc, and a consequent diminution of the volume of the sample of air when allowed to stand in the apparatus for a short time.

The NaOH used was a 40-per-cent. solution, and the pyrogallic acid was a saturated solution diluted to one-fourth its original strength.

The seeds employed were the ordinary cereals, and also *Eucalyptus globulus, Acacia melanoxylon, Cytisus laburnum, Setaria italica, Ricinus cambogiensis, Cannabis sativa,* and *Pinus insignis*—and the experiments were performed in four series.

1. The seeds used were tested as received from storage.

2. The seeds before being tested for their respiratory activity were dried in the oven for eight days at a constant temperature of 45° C.

3. Samples of the seeds after drying for eight days at 45° C. were further heated in the oven for three days. During the daytime the temperature of the oven was 100° C., whilst at night the temperature fell to about 70° C.

4. Some of the above seeds were still further heated to about 130° C., when all were killed, and the gaseous exchanges were again tested.

For each respiratory test a weighed quantity of each seed was passed up

* 'Annals of Botany,' 1908.

into the upper part of a narrow, perfectly dry test-tube, containing a known volume of air, over mercury. These test-tubes had been previously sterilised by dry heat in all cases, although with the thoroughly desiccated seeds this precaution is not really necessary, except as a means of drying the tube.

The tubes containing the seeds and mercury were set up vertically in a shallow dish of mercury, where they were kept for from 5 to 15 days. After this time had elapsed, samples of the contained air were drawn into the Bonnier and Mangin apparatus, in which their composition was ascertained.

Those seeds which were found to emit no carbon dioxide in their ordinary stored condition were not further tested for signs of respiration in the more completely desiccated state, but the quantity of moisture was ascertained in every kind of seed used. The relative amounts of water present in the seeds at different stages of desiccation are set down in a special table which follows the respiration tables given below. Throughout these experiments the seeds used were the freshest obtainable, and several analyses were made of each sample of air, the results tabulated below being the mean of these analyses.

Respiration of Seeds.

Kind of seed.	State.	Weight.	Volume of air.	Time.	Mgrms. CO_2 per grm. of seeds per day.	Percentage volume of O_2 absorbed per day.
		grms.	c.c.	days		
Acacia melanoxylon	As stored	2	5	6	0·005	0·15
Avena sativa	,,	6	18	7	0·0	0·07
Cannabis sativa	,,	5	4	6	0·0	0·3
Cytisus laburnum	,,	5	4	6	0·001	0·15
Eucalyptus globulus	,,	2	2	6	0·0	0·05
Hordeum sativum (barley)	,,	6	18	7	0·0	0·014
Setaria italica	,,	2	5	6	0·001	0·03
Pinus insignis.....	,,	5	5	6	0·0006	0·13
Ricinus cambogiensis.....	,,	5	7	6	0·0	0·10
Secale cereale	,,	6	15	6	0·004	0·15
Triticum vulgare	,,	6	18	5	0·13	2·5
Zea mais.....	,,	6	15	5	0·004	0·02
Acacia melanoxylon	Dried at 45° C. in dry heat for 8 days	2·0	8	5	0·0	0·14
Setaria italica	,,	1·2	6·5	4	0·0	0·02
Pinus insignis.....	,,	1·6	5·5	4	0·0	0·06
Secale cereale	,,	2·0	6	5	0·0	0·02
Triticum vulgare	,,	2·3	6	5	0·0	0·00
Zea mais	,,	2·0	5·5	5	0·001	0·12
,, ...	Further dried for 3 days at 100° C., dry heat	2·0	5·5	5	0·001	0·05

Table of Moisture contained in the Seeds.

Kind of seed.	Weight of seeds as stored.	Weight of seeds after 7 days at 45° C.	Weight of seeds after further heating to 100° C. for 3 days.	Percentage weight of moisture lost after 7 days at 45° C.	Percentage weight of moisture lost after 3 days at 100° C.	Respiratory activity of stored seeds.	Respiratory activity of seeds after 7 days at 45° C.	Respiratory activity of seeds after further heating to 100° C. for 3 days.
	grammes.	grammes.	grammes.					
Acacia melanoxylon.....	2·43	2·33	2·32	3·29	4·5	Slight	Nil	Nil.
Avena sativa.....	5·0	4·59	4·48	8·20	10·4	Nil	,,	,,
Cannabis sativus	2·37	2·27	2·21	4·20	6·8	,,	,,	,,
Cytisus laburnum.....	2·47	2·25	1·68	8·80	31·9	Very slight	,,	,,
Eucalyptus globulus.....	0·44	0·38	0·38	13·60	13·60	Nil	,,	,,
Hordeum sativum.....	5·0	4·70	4·52	6·0	9·6	,,	,,	,,
Setaria italica	2·41	2·24	2·18	7·0	9·5	Very slight	,,	,,
Pinus insignis	3·26	3·10	3·09	4·9	4·9	Extremely slight	,,	,,
Ricinus cambogiensis	5·05	4·89	4·72	3·2	6·5	Nil	,,	,,
Secale cereale	2·97	2·73	2·62	8·0	11·9	,,	,,	,,
Triticum vulgare.....	2·48	2·28	2·19	8·0	11·6	Strong	,,	,,
Zea mais	2·39	2·20	2·10	7·9	12·1	Slight	Trace	Trace.

Comparing the preceding tables it is seen that a feeble respiratory activity is shown by some of the seeds examined in the ordinary stored condition, which varies according to the seed and to the amount of moisture it contains. Oats, hemp, barley, Eucalyptus, and Ricinus showed no evolution of carbon dioxide, and the trace of oxygen absorbed may be the result of chemical oxidation or physical absorption.

Respiration was surprisingly active in the fresh wheat as obtained from the seedsman, although not more than 1/300 part of what it is in an active seedling. Further, the evolution of carbon dioxide ceased after a comparatively small degree of desiccation. Reference to the moisture tables shows that the percentage of moisture contained by the wheat was not relatively large as compared with the other seeds employed.

As might be expected from the fact that the seeds of *Acacia melanoxylon* are completely covered by a cuticle, which in the fully dried seed can withstand nearly 45 minutes' immersion in strong sulphuric acid without becoming permeable to water, the percentage of moisture eliminated by slow desiccation in dry heat is less marked than in any of the other varieties of seeds. The carbon dioxide evolved from the air dried seeds does not amount to 1/10000 of the amount evolved from an active seedling, and is, in fact, nearly within the limit of error, and may possibly be the result of oxidations taking place in the arillar appendages of the seeds.

In any case, all respiratory activity as evidenced by the gaseous exchanges completely ceased after the same seeds had been desiccated at 45° C. for seven or eight days. For purposes of control various dead seeds, fragments of dead wood, etc., were similarly tested in the air dry condition. In no case could any sign of an evolution of carbon dioxide be detected, and the fact that in some cases a trace of oxygen disappeared is not surprising, considering the structure of the materials tested, and their large bulk relatively to the amount of the enclosed air.

The above results indicate that respiration is not a function of completely dry seeds, nor even of seeds after a mild degree of drying, for only in one isolated instance, that of *Zea mais*, was there the faintest trace of any apparent respiratory activity present after remaining at 45° C. for one week. The amount of carbon dioxide produced in this case was less than one-millionth of that produced by an active seedling in the same time, and was evidently the result of the slow outward diffusion from the bulky seeds of carbon dioxide formed while in the air dry condition. The same applies to the traces of oxygen absorbed, as the gaseous relationships inside and outside the seed become equalised.

This is made even more evident by the fact that the greater number of the

fresh seeds in the air dry condition as obtained from the seedsman exhibited no respiratory activity whatever, although they contained quite appreciable quantities of water. Among these non-respiring seeds *Ricinus* is included, though in another species of *Ricinus*, Becquerel* states that an active interchange of gases does occur in the dried seeds, which he asserts to be a purely non-vital chemical action.

The experiments of Kolkwitz† carried out on barley illustrate the important effects produced on the respiration of seeds by the presence of moisture in the seeds. He found that—

1 kilogramme barley grains at summer temperature gave off 3·59 m. mg. of CO_2 in 24 hours when 19 to 20 per cent. of water was present;

1·4 m. mg. with 14 to 15 per cent. of water; and

0·35 m. mg. with 10 to 12 per cent. of water.

Becquerel‡ also discusses the effect of the presence of moisture in the seeds on the respiratory activity of the seeds in his extensive researches on the latent life of seeds, a short account of which also appears in the 'Comptes Rendus,' vol. 143, No. 26, December 24, 1906, p. 1177.

Summary.

The resting seeds of cereals such as wheat, maize, barley, oats, and rye all contain diastatic, fibrin-digesting, and ereptic ferments in appreciable amount. These ferments retain their activity without appreciable change in stored dry seeds for 20 or more years, that is long after the power of germination has been lost, which takes place in wheat after 11 to 16 years, barley 8 to 10 years, oats 5 to 9 years, maize and rye over 5 years. The life of the stored seeds is largely dependent upon the climatic conditions, a dry climate favouring longevity. Thus South Australian wheat lasts longer than that stored in Victoria, and still longer than that obtained from New South Wales. The difference is, however, not shown strongly until after the fourth or fifth year, South Australian wheat being still one-third germinable after 9 to 10 years, whereas wheat stored in Victoria had entirely lost its vitality by this time.

No relation was noted between the vitality of seeds and the persistence of enzymes in them, but since the enzymes persisted longer than the power of germination, the question as to whether germination could take place in the absence of any pre-existent enzymes remains to be answered. In any case

* 'Comptes Rendus,' vol. 143, 1906, p. 974.

† 'Ber. d. Bot. Gesell.,' vol. 19, p. 285, 1901.

‡ 'Ann. Sci. Nat., Bot.,' ser. 9, vol. 5, 1907.

no otherwise non-germinable seeds could be excited to germination by the addition of any kind of enzyme, and where the germination was feeble the addition of enzymes usually lowered the percentage germination and often delayed germination also to some extent.

The erepsin appears to be more abundant than the pepsin, but otherwise in the cases of all three ferments greater differences are shown between different samples of the same age than between different seeds, or between the same seeds of varying ages. Pepsin appears, however, to be more abundant in rye than in any other cereal, and is almost absent from maize. Dry oats, barley, and wheat can in part resist a temperature of 99° to 100° C. for 1 to 4½ hours; after 6 hours' exposure all are killed, but the ferments are apparently unaffected. All the ferments are destroyed after an hour's dry heat at 130° to 131° C. The pepsin appeared to be least (1 hour at 124° C.), the erepsin more (1 hour at 124° to 128° C.), and the diastase, especially of barley, most resistant to dry heat (1 hour at 124° to 131° C.).

Two days' exposure to liquid air, although it delays the subsequent germination and may also decrease the percentage, does not absolutely destroy any of the seeds tested and does not appreciably affect the ferments in any of the cereals. The dry diastase of barley is therefore able to withstand a range of temperature of $-200°$ to $+130°$ C. It is therefore thermally a highly stable chemical compound.

Many seeds, including all cereals, give off appreciable quantities of carbon dioxide when stored in the air dried condition, but others show no signs of respiration whatever. The respiration of air dried wheat is especially pronounced, but in practically all cases every sign of respiration ceases when the seeds are moderately desiccated, although in the case of large seeds like maize minute traces of carbon dioxide may continue to escape for a time.

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Croonian Lecture.—*The Functions of the Pituitary Body.*

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(Lecture delivered June 10,—MS. received July 22, 1909.)

The observation of P. Marie (1885) that the disease to which he has given the name "acromegaly" is associated with tumours of the pituitary body has caused this organ to attract the recent attention of pathologists to a greater degree than any other of the structures which were formerly classed together under the generic name "ductless glands." Since Marie's description of that disease, very many cases have been recorded, and in most of these the same association has been noticed.

The most striking sign of acromegaly is the increased growth of certain parts of the skeleton, especially the lower jaw and the extremities of the limbs, with hypertrophy of the connective tissue; indeed, the enlargement of the hands and feet is frequently the first change which calls attention to the existence of the disease, the patient finding that his gloves and boots are becoming too small for him. In the later stages there is dorsal kyphoscoliosis. Headache is a prominent symptom, polyuria is often present, and the eyesight is frequently affected. Acromegaly usually occurs in adults, often about middle age, although it may begin during adolescence. An allied affection—(pathological) gigantism—which occurs before normal